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THE TRANSMISSION OF *THEILERIA PARVA* BY TICKS

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(With 19 Figures in the Text)

INTRODUCTION

IN 1937 we reviewed briefly the investigations into the transmission of *Theileria parva*, the causal organism of East Coast fever, by the ticks *Rhipicephalus appendiculatus*, *R. evertsi* and *R. simus*. We proved that infective, unfed nymphae of *R. appendiculatus*, exposed to low, alternating and relatively high temperatures for 1, 2 and 3 weeks, transmitted East Coast fever when they were subsequently fed on susceptible cattle. There was no significant difference in the disease in our experiments from that observed under natural conditions. Ticks exposed to high temperatures, however, did not withstand the treatment so well as those exposed to the low temperatures. We referred to peculiar or mild forms of East Coast fever where Koch's bodies and small piroplasms were rare or absent; and to the disease, "turning-sickness" or "muthioko", associated with the blocking of the small capillaries of the brain by lymphocytes containing organisms indistinguishable from the schizonts of *T. parva*. *Hyalomma impressum* near *planum* was incriminated as a vector of *T. parva*.

We have continued our experiments according to plan and have studied the effect of low temperatures on the development of *T. parva* in engorged larvae of *R. appendiculatus*. We have also investigated problems of more immediate practical value suggested by administrative difficulties in the field. We show that *R. appendiculatus* fed on mild reactors to East Coast fever, when Koch's bodies were never numerous and small piroplasms could not be detected microscopically in blood smears, was able to set up a virulent form of the disease in susceptible cattle. Infected batches of *R. appendiculatus* have often failed to transmit East Coast fever, and we were unable to explain the apparent disappearance of the parasite. Evidence is now produced to show that *T. parva* does not always survive as long as the tick host under laboratory conditions. We have attempted to ascertain the earliest time when a tick can take up infection from a reacting beast; and whether the course of the disease can be modified by the route of infection and by the degree of tick infestation. Further attempts to produce East Coast fever from "turning-sickness" have failed. Epidemiological observations, however, indicate that at an early stage in the evolution of "turning-sickness" ticks might take up infection and be able to transmit East Coast fever. Experiments with

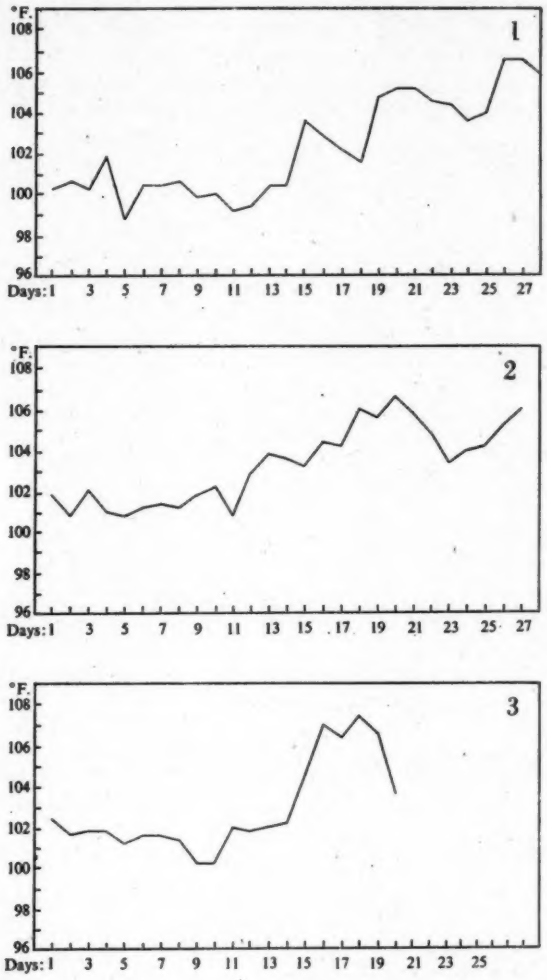
Hyalomma species other than *H. impressum* near *planum* have shown that *H. dromedarii* and *H. anatolicum* can, under laboratory conditions, transmit *T. parva* to susceptible cattle.

The effect of low temperature on *T. parva* in replete larvae of *R. appendiculatus*

In previous experiments on the effect of low temperature on the development of *T. parva* in the tick (1937), we used unfed nymphae which had been infected as larvae. The parasite in the nymphae, according to Cowdry & Ham (1932), would have developed to the stage of sporont-sporoblast and may be considered to have reached a rather passive phase of development in the tick which has not attached itself to a host. The relative inactivity of the parasite at this stage might account for the resistance to low temperatures. The earlier and more active forms of *T. parva* might more readily be affected by extreme cold. On this occasion, therefore, we exposed engorged larvae at different periods after the ticks had dropped off a reacting bovine whose blood was heavily infested with small piroplasms.

The engorged larvae of the first series of experiments were collected from bovine no. 2104 and divided into seven lots of 200 each. The first lot was used as a control, the larvae being put into an incubator within an hour or two after dropping off the host. The second lot was placed in a cold chamber in order to ascertain the effect of low temperature on the intra-erythrocytic and the free parasites in the gut of the tick. The remaining batches were first incubated for different periods as shown in Table 1, then transferred to the cold chamber for 3 days, after which they were returned to the incubator for moulting. The periods for the first incubation were calculated from those given by Cowdry & Ham, but adapted to the different conditions under which our ticks were reared. It was estimated that the usual development in an incubator for 2, 4, 6 and 8 days and exposing the ticks to cold at the end of these respective periods would give an idea of the effect of low temperature on the intra-epithelial, the zygote, the oökinete and the post-oökinete stages of *T. parva*. We should explain that larvae are normally incubated soon after they are collected off the host. They moult, on an average, in 8½ days; and it will be seen in Table 1 that despite an exposure of 3 days to a temperature of 4–6° C. (39·2–42·8° F.) in the cold chamber, the maximum period for moulting is 15 days. Cowdry & Ham pointed out that the rate of development of both ticks and parasites may be found to be very different from that which they described unless the environmental conditions were roughly parallel. They kept engorged larvae and nymphae at temperatures varying from 14 to 17° C. (57·2–62·6° F.) for about 2 weeks, and then put them in an incubator at 28° C. (82·4° F.) where they moulted 24 days after repletion. Our ticks were incubated at a temperature ranging from 25 to 27° C. (77·0–80·6° F.), the relative humidity varying from 50 to 60%, and the rate of evaporation, measured by a Piché evaporimeter, averaging 3·6 c.c. in 24 hours.

A continuous period of 3 days in the cold chamber was considered to be sufficiently long and severe in view of the results of preliminary trials, which



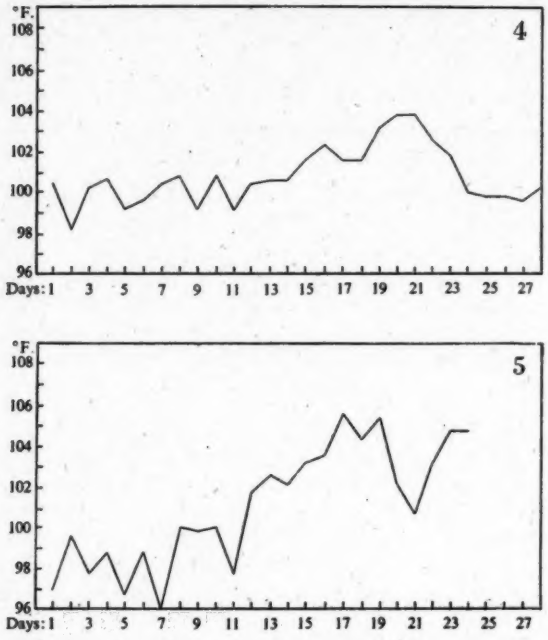
Figs. 1–3. Temperature reaction of bovine in first series of experiments. Infected by ticks. Fig. 1, first series no. 2, bovine no. 1451 died on 33rd day; Fig. 2, first series no. 3, bovine no. 833 died on 27th day; Fig. 3, first series no. 4, bovine no. 782 died on 20th day.

showed that 50% of the replete larvae died in that time, although we discovered later that, in some batches, the mortality was not always so high.

A second series of ticks was fed on, and collected from, bovine no. 9390, which also was suffering from East Coast fever. In this instance each lot

comprised 500 engorged larvae. Unfortunately the full series could not be used in subsequent feedings because of the shortage of suitable susceptible cattle at that time.

It may be observed in Table 1 that the second series of larvae survived the exposure to low temperature better than those of the first series; they moulted in a shorter time after their return for the second incubation, and the total number of days from repletion to moulting was decidedly smaller.



Figs. 4, 5. Temperature reaction of bovine in first series of experiments (continued). Infected by ticks. Fig. 4, first series no. 6, bovine no. 8658 died on 24th day; Fig. 5, first series no. 7, bovine no. 940, died on 24th day.

The nymphae which emerged from the moultings were kept at room temperature (18–20° C.; 64·4–68° F.) for not more than 2 months. They were fed on cattle whose history indicated that they were suceptible to *T. parva* but not necessarily free from *T. mutans*.

In Table 2 we give data on the number and the ages of the ticks fed on cattle and we summarize the results of infestation by the infective nymphae.

It is evident that *T. parva* in engorged larvae of *R. appendiculatus* is not destroyed, nor is its virulence affected, by exposure for 3 days to a temperature of 4–6° C., a temperature rarely encountered for such a continuous period in the inhabited areas of Kenya Colony. Exposure to this degree of cold for the time stated does not retard the evolution of the parasite from the

ingested intra-corpuscular piroplasm to the "oökinete" or "post-oökinete" phase described by Cowdry & Ham. The results of the experiments, so far, indicate that so long as the tick survives, the thermal conditions do not kill or weaken the parasite.

Only one bovine in the series of experiments failed to react to East Coast fever. This was no. 2103, which was proved to be susceptible on further test

Table 1

Ref. no.	1st incubation days	Cold chamber days	Survival %	2nd incubation days	Moulting days
			First series		
1	11	0	76	0	11
2	0	3	32	12	15
3	2	3	29	10	15
4	4	3	22	8	15
5	6	3	35	5	14
6	8	3	94	1	12
7	9	3	93	0	9
			Second series		
1	8	0	67	0	8
2	0	3	55	9	12
3	2	3	65	8	13
4	4	3	27	3	10
5	6	3	57	1	10

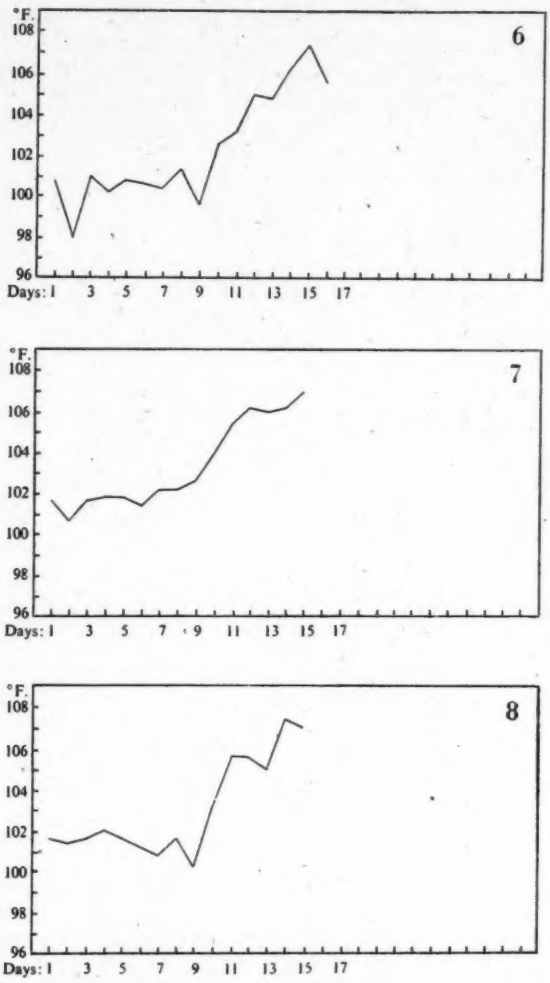
Table 2

Ref. no. of ticks	No. of bovine	No. of ticks		Age of ticks (in days)	Result of infestation*			
		Put on	Engorged		Reaction	1st rise of temp.	First piro-plasms	Death or recovery
				First series				
1	2463	200	170	59	+	13	17	25
2	1451	64	21	30	+	15	21	31
3	833	58	17	29	+	13	18	31
4	782	54	6	29	+	15	18	20
5	2103	70	25	26	–	Nil	Nil	–
6	8658	188	60	26	+	16	19	25
7	940	186	74	38	+	12	15	24
				Second series				
1	2464	334	122	47	+	10	13	16
2	2468	279	189	43	+	10	13	15
3	2467	327	129	43	+	10	13	16
4	2470	136	73	44	+	13	17	20
5	2466	284	102	45	+	10	14	18

* Figures in columns 7, 8 and 9 denote the number of days after infestation of the bovine with infected ticks.

with infective ticks. Bovine no. 8658 recovered in 25 days after infestation and, during the reaction which did not reach a temperature of 104° F., showed only a few parasites in the blood and the gland smears. Piroplasms in blood smears taken from bovine no. 782 were rare until the day of its death when they were fairly frequent; Koch's bodies in gland smears were numerous. The remainder of the animals in the first series, and all in the second series, died of typical East Coast fever.

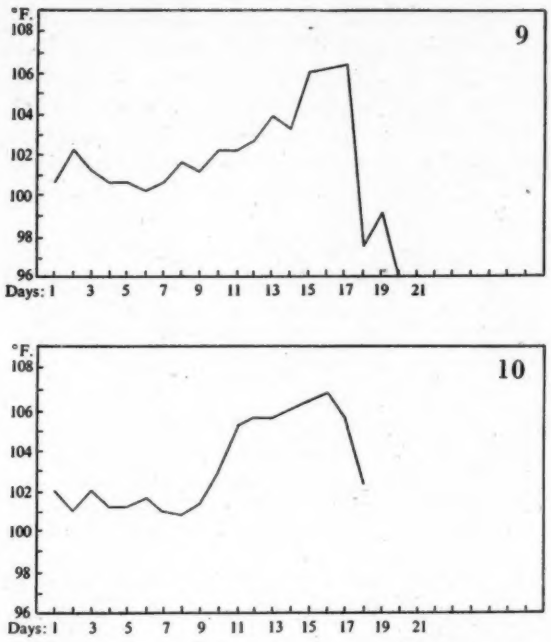
Other observations of interest in these results are (1) the difference in the course of the disease in the cattle infected by the first series of ticks and in the



Figs. 6–8. Temperature reaction of bovine in second series of experiments. Infected by ticks. Fig. 6, second series no. 1, bovine no. 2464 died on 16th day; Fig. 7, second series no. 2, bovine no. 2468 died on 15th day; Fig. 8, second series no. 3, bovine no. 2467 died on 16th day.

cattle infected by the second series of ticks. This is strikingly shown in the charts from which those for bovines nos. 2103 and 8658 of the first series are omitted. The temperature reaction is much shorter in the second than in

the first series and suggests a more virulent form of East Coast fever. (2) The incubation period in the first series is not less than 11 days whereas in the second it is usually about ten days except in the case of bovine no. 2470. (3) Small piroplasms were seen earlier in blood smears taken from animals in the second series than in similar smears from the cattle in the first series since the incubation period was shorter.



Figs. 9, 10. Temperature reaction of bovine in second series of experiments (continued). Infected by ticks. Fig. 9, second series no. 4, bovine no. 2470 died on 20th day; Fig. 10, second series no. 5, bovine no. 2466 died on 18th day.

It may be argued from the figures in Table 2 that these differences are due to the larger number of ticks used for the second lot of cattle or to the age of the ticks. In this instance, however, the differences are not solely due to the number of ticks but may, partly at least, be accounted for by the poor condition of the second lot of animals during the very dry spell of weather.

FEEDING INFECTED TICKS ON THE TAIL

Purvis (1937) suggested that infection of cattle with East Coast fever by feeding infected ticks on the tail might, like the pleuro-pneumonia vaccine given in the tail, be similarly less virulent than by the infection from ticks on the body or the ears. In accepting this suggestion, we put 300 infective

nymphae of *R. appendiculatus* in the tail brush of a susceptible animal which had been thoroughly dipped and hand-dressed prior to its being stalled in a tick-free shed.

The nymphae were put on bovine no. 2113 and prevented from escaping by a small bag tied over the tail-brush. Only two nymphae fed. The others died, possibly because the bag was tied too tightly or the site was unfavourable for this phase in the life-cycle of *R. appendiculatus*.

Bovine no. 2113 showed (Fig. 11) a rise of temperature to 104·6° F. (40° C.) on the 12th day after infestation. The temperature reaction lasted for another 13 days, and the bovine died on the evening of the 25th day from the time the ticks were put on. Gland and blood smears taken throughout the reaction contained typical Koch's bodies and small piroplasms. Post-mortem examination confirmed the clinical and microscopic diagnosis of East Coast fever.

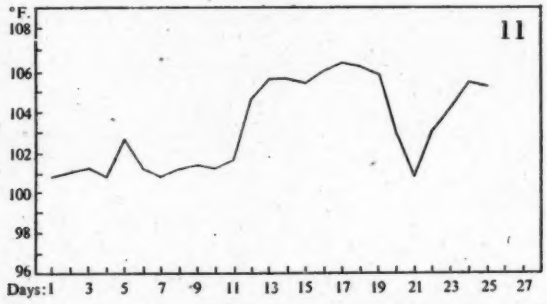


Fig. 11. Infected by ticks fed on tail brush; bovine no. 2113 died on 25th day.

In this instance, transmission of the disease by the tail route revealed no difference from that produced by feeding ticks on the ears of the bovine.

Transmission by *R. appendiculatus* fed on mild reactors

The occurrence of mild reactors to East Coast fever and recoveries from the disease is by no means rare in East Africa (Walker, 1927; Hornby, 1933; Mettam & Carmichael, 1936; Mettam, 1937; Daubney, 1938; Carmichael, 1939). It is not confined to native and low grade cattle but has been observed in high-grade herds on European farms.

Mettam & Carmichael and Daubney have also pointed out that in some natural cases it was difficult or impossible to demonstrate the presence of parasites. This feature of East Coast fever was observed in South Africa. Theiler (1907) referred to a statement by Robertson of Cape Colony: that East Coast fever may run its course with the total absence of *T. parva*, or perhaps with the presence of only a very small number of these parasites. Mettam quotes a case where the schizonts were rare in the gland smears of an animal which showed no clinical symptoms of East Coast fever. Walker had observed

that: "The reaction to natural re-infection is usually not severe and may escape notice in cattle not under close observation but sometimes a marked reaction and death occurred."

In the course of our investigations several of the susceptible animals gave reactions of different degrees of virulence. We were fortunate in having fed ticks on these animals because we desired to know the extent to which mild reactors could infect ticks and, by this means, disseminate East Coast fever. Walker had already claimed that the results of his experiments were of practical importance in connexion with the T-branding of cattle destined for transport work from infected to clean areas or *vice versa*. Lewis (1932) had also remarked that: "Not only can transport oxen convey uninfected ticks which may form a nucleus for a potential outbreak of East Coast fever, but it is possible that a percentage of the ticks may become infected during the reaction of the beast and transmit the disease to susceptible cattle in a clean area."

Bovine no. 2145 was infested with infected adults. The temperature (Fig. 12) began to rise on the 14th day. After reaching a maximum temperature of 105° F. on the 19th, the animal gradually recovered. Koch's bodies were numerous and piroplasms frequent or abundant from the 19th to the 23rd day, after which both forms of the parasite soon disappeared.

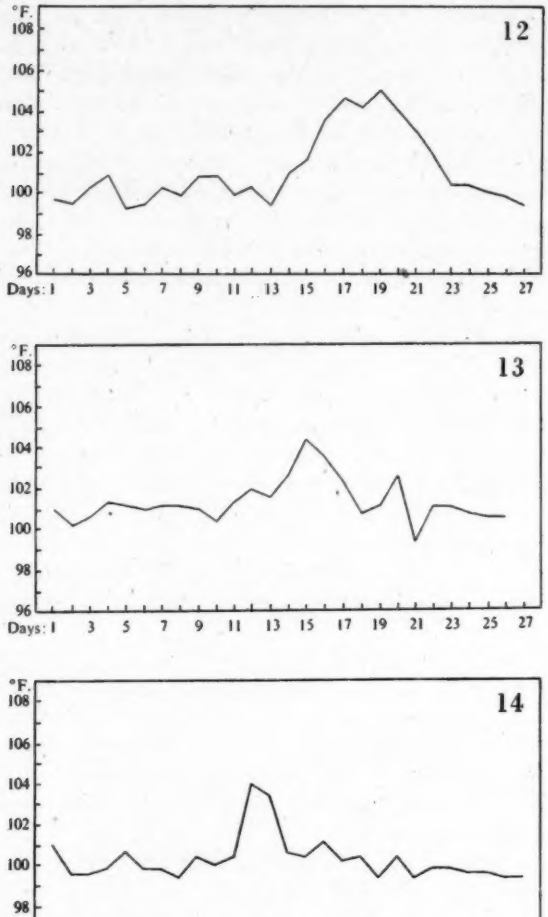
One lot of clean larvae was put on one ear of bovine no. 2145 on the 20th day from the original infestation by infected ticks. These larvae failed to feed and died. Another lot of clean larvae was fed on the other ear on the 24th day when only a few piroplasms were seen in blood smears and the temperature of the bovine had returned to normal. Only forty-three engorged larvae were recovered; and of these seventeen moulted and survived for feeding as nymphae.

The nymphae were placed on bovine no. X 9261. Three fed to repletion and fourteen died without attaching. The chart of this animal shows a short significant rise of temperature (Fig. 13) which might easily escape notice on a farm. The animal recovered. Koch's bodies in gland smears were numerous on the 21st day and rare at other times. The piroplasms in the blood stream did not reach a microscopic density throughout this mild reaction; and we were unable to ascertain their presence in the blood cells.

More clean larvae and a batch of nymphae were fed on both ears of bovine no. X 9261 on the 7th day of the incubation period. These ticks were to be used in experiments to ascertain the earliest period of reaction that the ticks would take up infection, an aspect of the subject to which we shall refer later. One hundred and fourteen nymphae which dropped off bovine no. X 9261 on the 3 days following the height of reaction to East Coast fever were allowed to moult and kept, as unfed adults, for about 4 months. They were then fed on bovine no. 3573 and fifty-six engorged females were recovered.

Bovine no. 8106 showed a temperature reaction (Fig. 16) typical of East Coast fever and died on the 28th day after infestation. Koch's bodies occurred in smears made from the ear and shoulder glands and they increased in

abundance as the disease progressed. Small piroplasms were numerous in the blood in the final stages of the disease. Post-mortem examination confirmed the clinical and microscopic diagnosis of East Coast fever.



Figs. 12–14. Mild reaction to East Coast fever followed by recovery and immunity. Fig. 12, infected by adult ticks, bovine no. 2145; Fig. 13, infected by three nymphal ticks, bovine no. X 9261; Fig. 14, infected by thirty-three nymphal ticks, bovine no. 1458.

Bovine no. X 9261 contracted a mild disease while bovine no. 8106 contracted a severe and fatal form of East Coast fever. This experiment again seems to support the view that the number of ticks with which a beast is

infested influences the course of the disease. Our experience and the records of our experiments do not coincide with this view. Whenever possible, we put batches containing not less than 100 infective ticks, and frequently more, on a bovine. The number of engorged ticks recovered are often very much smaller; the others are partly-fed or dead before repletion. The records of twenty experimental animals taken at random reveal that in one case only were more than a hundred replete ticks collected from a bovine infested with 200 ticks. In nine of the cases, not more than six engorged ticks were recovered; and the disease, though showing variations, caused the death of the animal. The remainder of the cattle contracted a form of the disease, with a short, typical or long temperature reaction, produced by not more than thirty ticks; and death followed the reaction.

The problem of mild East Coast fever is not well understood; but it is evident from the above experiment that ticks fed on a mild reactor can pass on a virulent form of the disease.

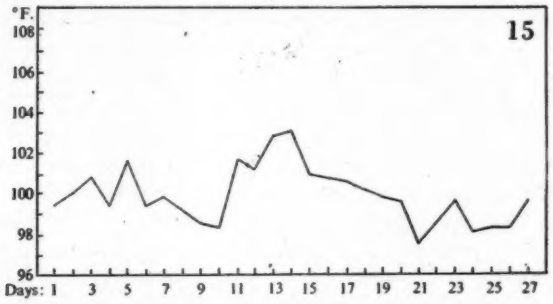


Fig. 15. Mild reaction to East Coast fever followed by recovery and immunity (continued). Infected by twenty nymphal ticks, bovine no. 2375.

Two more animals which were infested with 200 infeotive nymphae illustrate the difficulties which may be encountered in diagnosing the disease in the field; and indicate the necessity for strict precautions in attempts to control the spread of East Coast fever.

Bovine no. 1458 ran a normal temperature (Fig. 14) for 11 days after infestation. On the following 2 days, the temperature rose to 104° F. and then fell to 103·4° F. Afterwards it dropped to normal. Agamogenous Koch's bodies were seen in smears made from the ear gland on the 12th day, 104° F. They could not again be demonstrated. No piroplasms were detectable in blood smears. The bovine was reinfested with a larger batch of known infected ticks and proved to be immune.

Bovine no. 2375 was wilder than no. 1458 and its normal temperature (Fig. 15) was more irregular. It was infested with nymphal *R. appendiculatus* on the same day as no. 1458. When the temperature had reached its maximum of 103° F. on the 13th day, Koch's bodies appeared in the ear glands. Schizonts

were observed in the prescapular glands on the 15th day only, but no piroplasms were seen in smears made from gland tissue or blood. The animal recovered and, on further test, proved immune to East Coast fever.

The day of reaction when ticks first take up *T. parva*

This problem has arisen from enquiries in this and other territories of Africa: What is the earliest time in the reaction to East Coast fever that ticks can take in the parasite with the blood, and, after moulting, be able to transmit the disease? The question is intimately associated with the movement of stock and is a part of the broader problem of the spread of the disease.

Theoretically, *T. parva* reaches the tick only after the parasite has entered the blood stream, and thereby the gut of the feeding tick. We have shown that ticks may become infected when no small piroplasms are demonstrable by microscopic examination of the blood smears; and it is common experience to be unable to find the parasite in smears sent in for diagnosis from the field. Like Cowdry & Danks (1933), we found that in typical cases of the disease the piroplasms did not appear in the red blood corpuscles until 4 or 5 days after the first rise of temperature above 103° F. But it was often difficult to decide when the initial rise in temperature occurred, and we accepted the first rise as that temperature which was higher than the normal level recorded during an average period of 13 days, or when such an increase was accompanied by the appearance of Koch's bodies in the subauricular gland.

Theiler (1905) mentioned that ticks did not take infection during the incubation period, and not even during the first few days of the disease. In view of what we have already stated, it seemed that a biological test was the only sound criterion in a case of this nature.

Earlier in this paper we referred to clean *R. appendiculatus* which were put on bovine no. X 9261 7 days after its infestation with ticks infected with *T. parva*. The latter batch of ticks had dropped from the bovine before the clean nymphae were put on to feed. Engorged nymphae began to drop 4 days later or on the 11th day of the incubation period, and other lots were collected on each of the following 22 days. After these ticks had moulted, the following batches were fed on cattle susceptible to East Coast fever: (1) those which had dropped replete on the first 3 days following a rise of temperature (Fig. 13) of bovine no. X 9261; (2) a batch that dropped on the 4th day when the temperature began to drop from its maximum of 104·4° F.; (3) a number that was collected on the 5th day; and (4) a lot that dropped fully fed on the 6th day when the temperature was again normal. It will be recalled that the temperature of bovine no. X 9261 increased slightly (102° F.) but definitely on the 13th day and reached its maximum (104·4° F.) on the 16th day from the date of infestation by the infected ticks. These days represent respectively the 1st and 4th days of the reaction. It was on the 4th day that Koch's blue bodies were first seen in gland smears. They were observed in the blood only on the

5th day. No piroplasms were found by microscopic examination of blood smears.

The first lot of originally clean ticks, which dropped on the first 3 days of the disease, moulted to adults. Ninety-eight were then fed on bovine no. 2577. All attached and fed for several days, but only seventeen females fed to repletion. The thirty-two males were left on the beast for 20 days. The bovine failed to react to East Coast fever but proved susceptible to, and died of, the disease 2 months later.

The second lot comprised forty-five ticks which, as adults, were placed on bovine no. 899. Fourteen females dropped engorged; the remainder were males. This animal did not contract East Coast fever from the bites of these ticks. The same bovine was used for testing the third lot of ticks, which consisted of fourteen adults. All the ticks fed and eight engorged females were collected. Bovine no. 899 failed to react, but, on testing, died from a typical form of the disease.

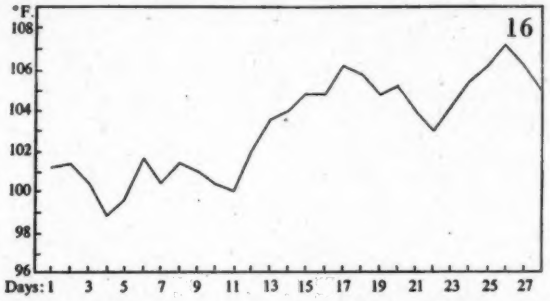


Fig. 16. Reaction produced by ticks infected on mild reactor, bovine no. X 9261; bovine no. 8106 died on 28th day.

The fourth lot of ticks was of 114 adults which were fed on bovine no. 8106 (Fig. 16). Fifty-five females dropped replete, and the beast died of typical East Coast fever.

Further trials with ticks that dropped off bovine no. X 9261 after the fourth lot resulted in transmission of the disease.

In this series of experiments, ticks took up the parasite with the blood after the 5th day of the temperature reaction.

Bovine no. 8106 was utilized to feed another series of clean nymphae. Its temperature (Fig. 16) increased to 102° F. on the 12th day after infestation with infected ticks, and to 103·6° F. on the 13th day. Koch's bodies appeared in gland smears on the 13th day which may be considered as the first day of reaction.

The clean nymphae were put on the bovine on the 4th day of the incubation period, and they dropped replete from the 9th to the 27th days inclusive. After moulting, the resultant adults from batches which had dropped on the first and the following 6 days of the temperature reaction of bovine no. 8106

were fed on susceptible bovines. The first five animals did not contract East Coast fever, although the ticks fed well. The sixth animal reacted and died.

These two series of transmission experiments confirm Theiler's statement that ticks do not become infected with *T. parva* during the first few days of the disease; and they show that the earliest time for infection, in these instances, was not before the 5th day from the first rise of temperature. In calculating the period in hours, we found that ticks dropping after 4 and before 5 complete days were infective. Thus, we may state on the evidence obtained, that 4 full days of the reaction may elapse before ticks are able to take up *T. parva* from the blood of a diseased bovine.

Longevity of *T. parva* in *R. appendiculatus*

We know that adult *R. appendiculatus* will survive 1013 days under laboratory conditions; and that nymphae will live 609 days without a meal. The natural environment in which these ticks normally remain when awaiting a host is more rigorous; and whereas the ticks reared and stored in the laboratory are sheltered and confined, those in the pastures are exposed to fluctuations of the weather and are likely to be more active. The longevity of the tick in nature, therefore, is probably less than that in the laboratory. The long period that the ticks live in the laboratory provide the opportunity to acquire precise information on the fate of *T. parva* in the tick host.

Theiler (1905) seemed to indicate the possible disappearance of *T. parva* in the ticks in the following statement: "It is now clear that the brown tick has almost completely vanished in an area which was once badly infected. The disappearance of the disease does not, however, coincide with the disappearance of the tick, brown nymphae and adults being found on all susceptible animals, and at a time when infection could reasonably be expected, namely, within 6 months after the death of the last animal on 24 January 1904. That the place did, however, not become reinfected was due to the fact that we did not allow a sick animal to graze. Hence the infected ticks which were not picked up by the cattle at that time probably were dead before the susceptible animals had arrived or had become purified." Du Toit & Viljoen (1929) remarked "...we know from former experiments and from the experience of the last 25 years, that the brown ticks will not live or remain infective for more than 14 or 15 months under veld conditions. And yet there are many cases on record where deaths from East Coast fever continued for 18 months, two years, or even longer, in spite of regular dipping and hand-dressing." Again, De Kock and his co-workers (1937) stated that while it is true that ticks have, under laboratory conditions, been kept alive for periods in excess of 28 months, it is extremely doubtful that a tick, under natural conditions, would survive so long; and even if it should, there is every reason to believe that an infected tick would, by that time, no longer be able to convey the infection to a susceptible host.

The duration of quarantine for East Coast fever is 15 months after the last

positive case. The period was ascertained from field experiments by Theiler (1904*a*) in South Africa, and based on three sets of findings; namely (*a*) the conditions under which the disease is spread by the brown and black-pitted ticks; (*b*) that it is only the intermediate stages in the life-cycle of the tick which takes up infection; (*c*) that the life-time of larvae and nymphae, and adults must be limited. He concluded from his experiments that an area, which at one time was badly infected with East Coast fever, does purify itself after a reasonably short time, and that, for the present, this period may be considered to be an average of 15 months.

It is obviously necessary to continue the work of Theiler and to determine, more accurately, the longevity of *T. parva* in laboratory-reared ticks and in ticks kept under natural conditions and to obtain information on the life of nymphae and adult *R. appendiculatus* in the different natural environments where the species thrives or manages to survive.

We observed that batches of infected ticks, used in experiments on transmission of East Coast fever, failed to produce the disease after they had been kept without a meal for about a year.

A very large number of larval *R. appendiculatus* was fed on a bovine reacting to East Coast fever and showing an abundance of parasites in the blood smears. The larvae were collected as they dropped, engorged, from the bovine. They were incubated in the usual way. When they had moulted to nymphae, the ticks were transferred to a cool store and used in transmission experiments at short, irregular intervals.

Table 3, summarizes the results:

Table 3

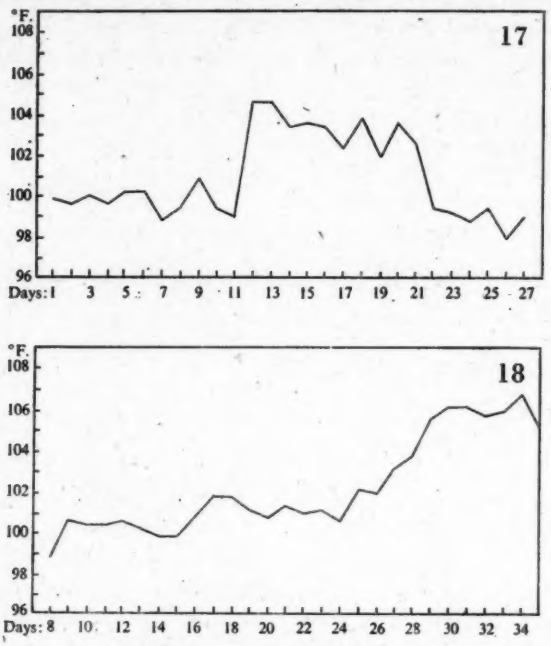
Age of tick (days)	No. of ticks fed	Result on bovine	Age of tick (days)	No. of ticks fed	Result on bovine
7	68	+	224	58	+
14	25	+	254	119	+
29	77	+(1)	284	5	+
42	73	+	314	33	+(3)
74	34	+	339	20	+(4)
76	33	+(2)	353	77	–
102	88	+	363	37	–
137	20	+	387	43	–
174	214	+	402	137	–
194	46	+	414	37	–

It will be observed that ticks up to 339 days old transmitted *T. parva* to the susceptible bovine on which they were fed, and that ticks from 353 to 414 days old failed to produce an infection of East Coast fever. The animals which did not react were tested with young, infected ticks and they proved susceptible.

The four bovines indicated by bracketed numbers in the third and sixth columns deserve further comment: bovine (1) had previously been infected with, and had reacted to, *Theileria dispar*. It recovered from the East Coast fever induced by the ticks, and on subsequent testing was found to be immune

to this disease. Bovine (2) also recovered from East Coast fever after a short, mild reaction with few schizonts and no visible parasites in gland and blood smears. It, also, proved immune in further tests.

Bovine (3) is of particular interest in that it gave a typical East Coast fever temperature reaction from the 12th day after the tick infestation to the 21st day. Thereafter, the temperature (Fig. 17) returned to normal and the beast recovered. Furthermore, gland and blood smears taken and examined daily failed to reveal any form of parasite other than an extremely rare *T. mutans*. The animal was tested by a large number of infective ticks and did not



Figs. 17, 18. Fig. 17, age of infective ticks=314 days; bovine no. 7437, recovered and immune. Fig. 18, age of infective ticks=339 days; bovine no. 6224 died on 35th day.

react again. We had also put clean larvae on one ear of this bovine in order to repeat our experiment on the earliest time in which the ticks take up infection. Some of the batches of the resultant nymphae transmitted East Coast fever (Fig. 19) and thus confirm the immunity test, that this bovine contracted the disease from ticks 314 days old.

Bovine (4) also is interesting. It reacted to and died of East Coast fever, as shown by the numerous Koch's bodies, piroplasms in smears taken during the temperature (Fig. 18) reaction, and by post-mortem examination. The incubation period, however, was much longer than usual and lasted for 25 days.

These two cases suggest that *T. parva* loses its potency in the ticks at about this stage of their life off the host. Reichenau noted that infection was lost at about a similar period in adults sent to him as engorged nymphae from the Veterinary Research Laboratory, Kabete. The negative results obtained from older ticks accentuate the significance of these unusual cases; and it may be of value to investigate still further the fate of *T. parva* in brown ticks kept unfed for the periods indicated.

Our tests with a series of adult *R. appendiculatus*, infected as nymphae, have not yet been carried out. Our records, however, show that adults up to 280 days old transmit the disease while those older than 320 days do not convey infection.

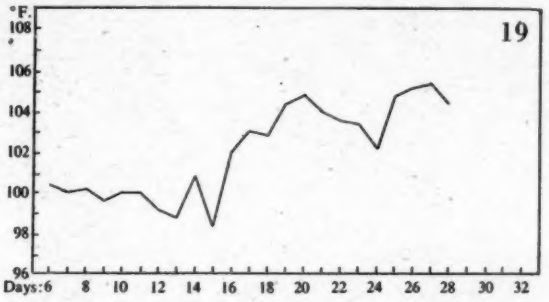


Fig. 19. Reaction produced by ticks infected on bovine no. 7437 in which no parasites were demonstrable; bovine no. 3573 died on 28th day.

EAST COAST FEVER AND "TURNING-SICKNESS" (MUTHIOKO)

In attempts to break down the immunity of East Coast fever by feeding ticks and to produce "turning-sickness" in the laboratory, we infected a number of animals with a series of large batches of *R. appendiculatus*. The bovines used had recovered from East Coast fever several years before from reactions of varying severity.

So far, we have failed to reinfect a recovered beast; and we have not succeeded in producing a case of "turning-sickness" experimentally. Neither have we been able to confirm our first experience of transmitting East Coast fever by ticks fed on an animal suffering from "turning-sickness". It is noted, however, that Mettam (1937) succeeded in recovering *T. parva* from both acute and chronic cases of "turning-sickness" in Uganda, and that ticks from such cases transmitted East Coast fever to susceptible cattle. Carmichael (1939) continued Mettam's investigations and reported: "All attempts at transmission completely failed, but study of the post-mortem lesions in a number of cases suggested a close connexion with East Coast fever." Carmichael also accepts, from his findings, that "turning-sickness" can be caused by *Trypanosoma theileri* as well as by species of *Theileria* and considers it necessary to reorientate the conceptions in regard to the etiology of the disease.

Whilst we recognize that the turning symptom in "turning-sickness" is not pathognomonic of *Theileria* infections, we wish to make it clear that we are dealing with that form of "turning-sickness" associated with the invasion of the brain tissue with Koch's bodies.

We consider the apparent relationship of East Coast fever and "turning-sickness" of special significance; and in envisaging each aspect of East Coast fever as part of a wider problem in Kenya Colony, we have taken advantage of every case of "turning-sickness" to study the nature and course of the disease in the field. A small number of cases was brought to the laboratory for infection of ticks and for detailed observation. One of us (W.F.) had an opportunity of examining herds of cattle, many of which developed symptoms of the disease soon after their release from an East Coast fever testing and quarantine station.

The history and nature of these outbreaks in the field together with post-mortem examinations at Kabete have led to the formulation of a hypothesis that may stimulate further research into the transmission, the etiology and the pathogenesis of "turning-sickness" and into the connexion between what may be considered to be two manifestations of the disease caused by *T. parva*. This hypothesis may also offer an explanation of the apparent conflicting results of the transmission of East Coast fever by ticks previously fed on "turning-sickness" cases.

Information on many cases of "turning-sickness" at Machakos, Kenya Colony, indicates that the disease occurs after the wet seasons. In the particular outbreak investigated by Fotheringham in 1939, it was after the "long rains" of May and June; but local evidence pointed to its prevalence either after the "long rains" or after the "short rains" in September. Its incidence may, however, merely coincide with the greater number of cattle passing through the East Coast fever quarantine station at these periods when the supply of grazing is abundant.

European traders with long experience of the disease maintain that "turning-sickness" appears at about the 6th week after the admittance of cattle to the quarantine station. These cattle are usually acquired in areas endemic to East Coast fever.

Out of 311 cattle examined at the Machakos quarantine in July 1939, one died of East Coast fever 26 days after admission; eight died of "turning-sickness" in the 7th and 8th week of the quarantine period. Another sixteen died of "turning-sickness" after their release and between the 8th and 16th weeks after their arrival at the quarantine station.

A number of cattle susceptible to East Coast fever and to "turning-sickness" were exposed with the released cattle sent to two adjoining farms in the Machakos district. They were exposed for 3 months. None contracted East Coast fever or "turning-sickness". It seemed, therefore, that both diseases from which the cattle died were picked up prior to the transfer to the farms, and that they originated in the quarantine station or in the reserve

where they were purchased or en route to the station, all three being endemic East Coast fever areas and presumably infested with disease-bearing ticks.

A cursory examination of the cattle at the quarantine station, revealed clinical evidence of East Coast fever in the form of swollen subauricular, prescapular and precrural glands; and the affected animals showed signs of recovery from the disease. A small number showed symptoms of "turning-sickness". Further examinations made it possible to separate the sick beasts into groups:

(1) A few that were brought to the laboratory. These had definite, clinical symptoms of "turning-sickness". Smears from blood, glands, spleen, brain and other organs contained small piroplasms and Koch's bodies. Post-mortem also revealed typical lesions of active East Coast fever; and the characteristic haemorrhagic lesions, of "turning-sickness", in the brain.

(2) Another lot suffering from clinical "turning-sickness" which, at post-mortem examination, revealed unmistakable evidence of recent East Coast fever infection in that the lesions of the fourth stomach and kidneys were healing and the lymph glands were swollen. No parasites could be demonstrated in the blood, lymphatic system, spleen and several other organs; but Koch's bodies were found in the brain lesions.

(3) A further group of "turning-sickness" cases where post-mortem examination disclosed lesions of a chronic nature, such as fibrosis and cavitation of the brain. No piroplasms or Koch's bodies could be found in the brain or any other organs, and anatomo-pathological evidence of East Coast fever was absent.

These epidemiological and pathological observations indicate that infection of ticks from "turning-sickness" could, normally, be successful only from such animals as we have put into the first group. These are acute cases and, unfortunately, seldom obtainable early enough for laboratory experiments on transmission. The second and third groups consist, respectively, of subacute and chronic cases, those of the former being met with most commonly in the field. The grouping is, of course, an arbitrary one; and there must be gradations from one group to the other. It is conceivable that even an early stage in the second group could provide parasites for ingestion by ticks and, thereby, be transmissible to the next instar for infection of cattle with *T. parva*.

In discussing the pathology of East Coast fever in Uganda, Mettam & Carmichael (1936) stated that in the great majority of cases, the nervous system is normal, and, to this extent, agreed with Steck (1928) and Cowdry & Danks (1933). In one case, they found schizonts (Koch's bodies) in the cortical smears of the brain whereas Cowdry & Danks state: "We observed Koch's bodies in the cerebral cortex in only two out of the twelve animals which had them in other parts of the body." We also have examined tissues of the brain from East Coast fever cases and have observed that if sections of the brain, cut in such a way as to include vessels of the choroid plexus, are examined, vascular changes fundamentally similar to those seen in the vessels of other organs, e.g. kidney,

are present. These changes consist of perivascular lymphocytic infiltrations with Koch's bodies and an increase in the number of white cells within the lumina of the affected vessels. These cells, also, carry Koch's bodies.

Is it not possible that the extension and intensification of this process in the brain is responsible for "turning-sickness" and that the pathological changes in this disease may, hypothetically at least, be considered as residual lesions of East Coast fever?

For the time being, we feel disposed to look upon "turning-sickness" or "muthioko" as a cerebral infection by *T. parva* which probably occurs when an animal partially resistant to East Coast fever is suffering from a second attack under exposure to massive infestation by infected ticks.

Discussion

The main object of our investigations into the transmission of East Coast fever by ticks is to seek, in this direction, an explanation of the different manifestations, and outbreaks, of the disease in order to assist in the formulation of a sound policy of administrative control.

We have endeavoured to provide accurate and more detailed information to complete the general field observations made in this, and other, territories; and to continue to study the disease transmitted by ticks under the different conditions to which they are subjected when feeding on an animal and during the period of their existence off the host.

Our laboratory investigations are not sufficiently comprehensive to warrant definite conclusions which may, with absolute assurance, be applied to field conditions but they contribute to a clearer understanding of the epizootiology of East Coast fever.

The distribution of the disease has been associated with topographical and climatic conditions. Its low incidence, or absence, in the high plateaux and districts in South and in East Africa has been attributed to the effect of cold temperafures on the common tick vector, *R. appendiculatus*. The rare occurrence of the disease in the dry plains and desert areas has been ascribed to the inability of the tick to thrive on account of the heat and dryness.

Van Saceghem (1930), however, found in the Ruanda district of the Belgian Congo that *Rhipicephalus* ticks which are vectors of *T. parva* did not transmit East Coast fever at altitudes above 8000 ft., and that when healthy cattle from these high levels were brought down to about 5000 ft., they contracted the disease and died. During the hot, dry season at Ruanda, ticks are excessively numerous. Van Saceghem stated that the heat factor evidently affects not only the number of ticks, but also the virulence of the organisms inoculated by them; and that the reverse is evidently equally true. He believed that it was the low temperature obtaining on the mountains and high plateaux, where the temperature may reach freezing-point, that prevented the ticks from propagating the disease.

We have not completed the study of the influence of high temperature and dry atmosphere on *T. parva* in ticks. The virulence of the parasite is not affected when its unfed nymphal host is kept for a week in a thermal chamber where the temperature is 38° C. (100·4° F.), the relative humidity from 40 to 52%, and the daily rate of evaporation is 6·2 c.c. by the Piche evaporimeter. The ticks themselves do not live for long under these conditions. The parasite, however, does not seem to be affected by this environment and persists so long as its host survives the exposure. When the development of engorged larvae of *R. appendiculatus* is accelerated by more moderate heat (25° C. or 77° F.) and moulting takes place in about 6–9 days, the parasite seems to adapt itself to the relatively rapid metamorphosis of the tick; and its virulence in cattle is not modified. Extremely warm and dry conditions may perhaps alter the virulence of *T. parva*, but we feel that such an environment would kill off the ticks first and so reduce the chances of the parasite in reaching a vertebrate host.

The influence of cold on the parasite as it develops in engorged larvae is negligible if the natural environment in which the tick lives is not more severe than the conditions to which those in our experiments were subjected. Here again, the temperature affects the rate at which the tick undergoes its development. Moulting is retarded; but the disease transmitted by infective ticks exposed to the low temperatures indicated in our series of experiments was not different from the usual, fatal forms of East Coast fever.

We are inclined to the opinion, therefore, that so far as the influence of temperature is concerned, *T. parva* will survive for as long a period as the tick-host.

This view is supported by field observations; and we associate to a large extent, the relative absence of East Coast fever in the high altitudes (7000 ft. and upwards) with the inability of *R. appendiculatus* to establish itself widely because of the cold; and not to the attenuation of *T. parva* in the tick. For instance, the disease occurs sporadically on the Kinangop plateau (7400 ft.), in the Limuru (7000 ft.) and the Molo (9000 ft.) districts of Kenya Colony. It runs its normal course and results in the death of the animal. The outbreak, however, soon dies out; and the losses are not very heavy.

The lowest average minimum air temperature in the first and the second areas is 37° F. (2·8° C.) in the coldest months of July and August. It is about 40° F. (4·44° C.) throughout the year in the third area. These low temperatures are recorded only for an hour or two before sunrise; and the rest of the day is usually much warmer. The temperature within the grass cover and of the top soil, where the ticks live, is not so low. It is evident, therefore, that the development of *T. parva* in the bovine and in the tick is not always affected by the cold at these altitudes. But tick surveys have shown that *R. appendiculatus* is not a common tick in these areas and, undoubtedly, accounts for the low incidence of the disease. The scarcity of the tick would appear to explain also why the disease does not spread readily and why the outbreaks are not severe.

R. appendiculatus does exist at these, and at higher, altitudes but is restricted to the sheltered localities; or it may have been brought on to farms by cattle moving from lower districts. These small infested "pockets" are known to be potential and actual sources of infection, but are often avoided for long periods once an outbreak has been traced to them.

We have shown that unfed nymphae and adults can live for over two years under favourable, artificial conditions but that *T. parva* in such ticks died before the completion of one year. In other words, the parasite did not live as long as the tick. It seemed that some change began to take place after 284 days and that the parasite had lost its ability to produce East Coast fever at, if not slightly before, the expiry of 353 days. The age of infected ticks, therefore, offers a further explanation to so-called peculiar outbreaks of East Coast fever and to cases of recovery from the disease. The disintegration or disappearance of the parasite substantiates, in principle, the safe period of quarantine established for East Coast fever by Theiler early in this century. It will be recalled that, in collaboration with Stockman, Theiler (1904*a*) concluded from the results of his field observations that an average period of 15 months would suffice for the cleansing of an area badly infected with East Coast fever. Theiler added that further experiments which were at that time in progress might show that the cleaning up of infection could be achieved in a shorter time. He seemed to suggest, from his general observations, that the period would not be less than 8 months. In 1905 he explained that the additional experiments were only partially carried out and that the principal problem was "whether the purification of the ground was due to the disappearance of the brown tick or to losing its virulence by the length of time which elapsed before the progeny could have become infected". This idea does not coincide with our interpretation of Theiler's remarks quoted earlier in this paper. But it is clear that he attributed the disappearance of infection by the opportunities offered to infected ticks to clean themselves and depriving clean ticks from gaining access to diseased animals. Reinfection was prevented by removing animals as soon as they showed a rise of temperature, and by allowing non-susceptible animals to graze over the infected area so that infective ticks could feed on them and thus get rid of parasites. By employing this method, cattle could safely be returned, after a lapse of a year, to the land where the infection started. The period of 15 months of quarantine was based on experiments where enclosed, infected pastures were kept free of all stock.

The results of our experiments on the longevity of *T. parva* in ticks, which were protected from adverse conditions, throw light on the disappearance of the disease in nature when it cannot be attributed to the absence of the ticks or to the cleansing of infected ticks by feeding on non-susceptible animals. They point to the possibility of reducing the accepted period of 15 months' quarantine if the farmer assisted with full cooperation, made every effort to protect his farm and herds, and persisted in reducing tick infestation. It is

first necessary, however, to repeat the experiments, to ascertain the longevity of *T. parva* in adult *R. appendiculatus*, and to determine the fate of the parasite in ticks under different natural conditions in East Coast fever infected areas.

The occurrence of mild East Coast fever, recovery from the disease and subsequent immunity to it, is interesting. It has revived the problem of the different virulence of the *Theileria* species of organisms, and it gives rise to speculation of ideas on immunity. This form of East Coast fever has been reported from European-owned farms and from native reserves. We have had it at the Laboratory. It occurs in adult cattle and in calves. But we have also produced severe and chronic cases which recovered. In fact, the temperature curves and the frequency of parasites in positive transmissions show a wide range of variations which do not fall into groups according to any particular factor or factors. Many suggestions have been made in an attempt to explain these mild cases and outbreaks; and we feel that they have been accepted with insufficient precise information, on the one hand, and without due consideration of the circumstances involved on the other.

Lounsbury, Theiler, Walker and ourselves have shown that death from East Coast fever usually followed whether the number of infected ticks fed on the beast was large or small. Purvis (1937) commented on the theory put forward in the *Tanganyika Annual Report* (1932) where Hornby (1933) wrote: "It has been suggested that this calf mortality (in areas where 60–70% of the calves succumb to East Coast fever) is in direct ratio to the degree of tick-infestation and that by weekly dipping without hand-dressing this infestation is inhibited so that in an enzootic area the calves become mildly infected by the ticks which persist in the ears and around the anus and immunity is attained with a loss possibly of 5–10%."

There may be, in nature, conditions that make it possible for an animal to combat infection produced by a small number of infected ticks: but in the absence of definite evidence of such, we cannot lightly dismiss the results of experiments which show that, ordinarily, the disease transmitted by even one or two ticks runs the usual course and terminates in death. It seems to us that if the term "light infestation" is applied to the density of ticks in the pastures rather than to the presence of a few tick vectors on the cattle, then it would be possible to explain the low mortality, at least partly, by the reduced chances that the ticks would have in reaching a host. It is not that the animals contract a mild disease through the medium of a few ticks, but that the small number of infected ticks gain access only to a few cattle and, in this way, account for a low mortality. Cattle from enzootic areas in Kenya Colony are not necessarily immune to East Coast fever. Adults purchased in such areas and passed into the infected quarantine paddocks not infrequently contract the disease for the first time, and die.

Hornby was aware of other factors which might have an influence on the occurrence of East Coast fever. In the *Annual Report* to which reference has

been made, he wrote: "There appears to be a seasonal and an annual variation in the incidence of this disease due no doubt in some measure to the climatic conditions affecting the development and propagation of the tick, and in part to the stock density. This is a portion of the problem, the whole of which requires further investigation." We agree that there appears to be a seasonal and an annual variation in the incidence of disease which is influenced, also, by stock movements, the frequency of dipping and the tick activity. Tick activity is not always so changeable in some enzootic areas as it might be in others. Theiler (1904*b*) and Stockman showed what influence the season had on the development of East Coast fever in herds of cattle exposed to a heavy tick infestation in a paddock at Nelspruit in South Africa. Out of eighty-nine animals turned into this paddock, only one recovered from the disease. An analysis of the observations revealed that the earliest infection contracted was in January and February: there was little variation in March and April: delay was noticeable in June and thereafter. The longest period of exposure required was in November and December. Theiler and Stockman remarked: "It is rather a startling observation that infection was so long retarded, 44 days in these 2 months, during which the temperature is so favourable to the development and moulting of ticks. A careful examination of the cattle, however, during these periods was constantly made and it revealed the remarkable fact that the brown ticks were the reverse of plentiful." This is attributed to the burning of the veld in the winter; and it indicated that "the rapidity of infection is in direct relation to the growth of the pasture". They concluded that the greater the number of ticks which infest a beast, the more is the chance of there being pathogenic ones among them. Accordingly the chances of rapid infection are greatly increased when the grass is long. It will be noted that the appearance of the disease in this case also is dependent upon the opportunity for ticks to reach a host in the first instance, and upon there being infected species among them in the second.

The naturally infected tick fauna will gradually become clean in enzootic areas where the movements of stock are restricted. The enzooticity depends on the calf population and on the introduction of susceptible adults. It is conceivable that a low birth-rate and high calf mortality due to common intercurrent diseases and other adverse circumstances will accelerate the cleansing of indigenous ticks; and that when calf production is later improved in the restricted area, cases of East Coast fever would be few or absent. Such a state of affairs might develop and make it appear that the calf mortality in a reputed enzootic area is low. This, together with a long dry season and a period of tick inactivity, may contribute to the impression, or suggestion, that light infestations of cattle by *R. appendiculatus* are responsible for the low mortality of calves.

We cannot offer a satisfactory explanation of the mild cases of East Coast fever; but we have been able to demonstrate that *T. parva*, recovered by feeding ticks on these reacting animals, is reinvigorated, and can cause a severe

reaction when transmitted by the ticks of the next instar. The mild form of the disease may easily escape notice in the field, and, in some cases where the temperature indicates East Coast fever, microscopic evidence may be absent. Nevertheless, infection in a virulent form may arise from such sources possibly after a lapse of several weeks or months, and the recurrence of the disease on a farm may be due to previous unidentifiable cases.

According to the experiments which we have carried out ticks can continue to feed for a maximum period of 4 days from the commencement of the temperature reaction without becoming infected with *T. parva*. Ticks dropping off a diseased animal before the 4th day, therefore, will not transmit East Coast fever after moulting to the next stage of its life-cycle. This information provides a means of assessing the margin of safety or danger in driving cattle from East Coast fever areas, through clean country, to places of slaughter; it allows for some relaxation of the permit and quarantine systems in cases of emergency, and when the movement of stock would occupy not more than 4 days. The temperature of all animals should, of course, be ascertained immediately before the movement, and any beast showing signs of reaction should not be released from the collecting paddocks or farms. This measure should, with justification, be resorted to only in extreme necessity and when facilities for a temperature boma, or efficient dipping, are not available.

When the movement entails a trek, on foot, of over 4 days, the normal precautionary steps have to be taken. We need not elaborate on the requirements of temperature camps and on the restrictions imposed by the laws relating to East Coast fever. It will suffice to say that animals released under the conditions prescribed by the recognized veterinary authority are usually free from risks of infection at the commencement of the journey. But in countries where much of the land is unfenced, where illicit or uncontrolled movement of stock cannot be prevented, and where the moving animals may wander away from the stipulated routes, there is always an element of risk that the cattle moved for slaughter may pick up infected ticks and contract East Coast fever.

The usual minimum period of incubation is 9 days; the average about 11 or 13 days. For 9 days, therefore, these cattle could traverse stretches of country without much danger of their dropping infective ticks and being responsible for the spread of the disease. And as ticks which drop off in the first 4 days of the reaction are also free from the causal organism, the safe period, as far as the land over which the cattle travel is concerned, can be extended to 13 days.

Summary

1. The development of *Theileria parva* in engorged larvae of *R. appendiculatus* exposed to a temperature of 4–6° C. for 3 days at intervals of 2, 4, 6, 8 and 9 days after repletion is not retarded; and it appears, from previous experiments and those now described, that, as long as the tick survives, climatic conditions do not kill or weaken the parasite.

2. The virulence of East Coast fever transmitted by ticks fed in the tail brush did not differ from the disease conveyed by ticks infesting the ears.

3. Reference is made to records of mild reactions to East Coast fever when the parasites are either rare or absent; and instances are given of such reactions followed by recovery in experimental animals. Although the mild form of the disease seems associated at times with light tick infestation, it is proved that a few infected ticks also transmit a fatal East Coast fever. It is shown also that ticks fed on a bovine during a short or mild reaction can produce a virulent form of the disease in susceptible animals, and that mild reactors acquire an immunity to the virulent disease.

4. The experiments which are described indicate that ticks do not become infected with *T. parva* from the blood of a bovine for the first 4 days of the reaction period.

5. Evidence is produced which strongly suggests that *T. parva* tends to disappear from infected hungry ticks kept under laboratory conditions for about a year or more. The age of the tick would appear to be an important factor in the transmission of East Coast fever.

6. Attempts to break down the immunity to East Coast fever, and to produce "turning-sickness", by massive infestations of ticks were unsuccessful; and further experiments on the transmission of East Coast fever by ticks which had fed on animals suffering from "turning-sickness" did not confirm earlier positive results.

These conflicting results may be explained by the observations that "turning-sickness", associated with the blocking of the small capillaries of the brain by lymphocytes containing schizonts indistinguishable from the Koch's bodies of *T. parva*, may be divided into three stages, namely, acute, subacute and chronic; and it is suggested that infection of ticks with *T. parva* from "turning-sickness" could normally be successful only from animals in the acute and early part of the subacute stages since piroplasms disappear from the peripheral blood stream in the more advanced cases of "turning-sickness".

7. The opinions of other workers on East Coast fever are discussed in the light of the experiments carried out at Kabete and of field experience in Kenya Colony. It is explained how the results can be applied to the movement of cattle from endemic areas over country free from East Coast fever.

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OBSERVATIONS ON NATURAL POPULATIONS OF THE BODY LOUSE, *PEDICULUS HUMANUS CORPORIS* DE G.

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(With 6 Figures in the Text)

Introduction

The materials for this paper were obtained from three sources: (*a*) the examination, on successive occasions during the period December 1939–April 1940, of volunteer subjects, mainly from common lodging houses in east London, prior to their treatment with pediculicides, (*b*) the examination, during April 1940, of large numbers of men from common lodging houses, Salvation Army hostels, night refuges and other sources, in a search for further suitable subjects for control experiments, and (*c*) the study of the process of infestation of shirts issued to selected volunteers and worn continuously over a period of 10 days. The material from the first source is largely selective, since infested volunteers were specifically invited; that from the second source is more representative of the population of cheap hostels, since no emphasis was laid on the presence of lice, the men being induced to submit to examination by the offer of payment. It is, however, obviously not a random sample since it represents volunteers, and neither it nor the source (*a*) material should be used as a measure of the frequency or degree of infestation normal to the corresponding group of the city population.

The full records on which this paper is based are very bulky; they are filed at the Cooper Technical Bureau, and are available for reference by interested workers.

Altogether, 263 sets of under-garments were examined, of which 34, 13%, were free of lice. The remaining 229 sets represent 151 men, some of whom were examined on more than one occasion (source (*a*) material). Of these 151, 15% had counts, on first examination, of over 100 lice, 7% over 250, and 3·3% over 500, 2 being over 1000.

Definitions

'Environment': the term has been confined in this paper to the physical environment of the infested subject.

'Habitat areas': this term is restricted to the different areas of the garment surface defined in the text.

'Territory areas' consist of two opposed surfaces. The first territory area is comprised by the skin and the inside of the first garment, the second by the outside of the first and the inside of the second garment. The third area is regarded, for purposes of the present study, as being one surface only, since examinations of the outer clothing were not made.

'Migration' and 'invasion' distinguish between the movement of lice from the outer clothing and from the 'environment' respectively of the infested subject.

'Native' lice are those which have hatched from eggs laid on the undergarment in question.

The consistency of infestation of individuals

The louse counts obtained for common lodging-house inmates consist of two groups, (*a*) 'original' counts, i.e. the first count on each new subject, and (*b*) several repeat or 'initial' counts on some of the men over the next few months. Most of the records, in the latter group, are due to a series of counts on certain individuals who were used repeatedly for louse control experiments, 2–4 weeks being allowed to elapse between one experiment and the next. The first count for each successive experiment, prior to treatment of the subjects, has been taken as an 'initial' count.

It became evident during the examinations that the infestation of an individual, observed from time to time, tends to remain within a given order of magnitude—his louse population level. Naturally this consistency was liable to interruption, from such causes as a recent change of garments being examined, or a recent visit to the municipal delousing station, but it was usually possible to predict the infestation level on repeat counts. Table 1 gives the counts made on nine subjects who were examined on five or more occasions.

Since single abnormally large counts swamp the average for the remainder, and also because of the wide fluctuations at high densities of population, the standard deviation has also been calculated as that of the logarithms from the mean log (Williams, 1937, 1940). By this method, which uses the geometric mean, the individual groups of counts fall into more clearly defined classes than when the arithmetic numbers are used. The geometric mean is, however, in this case a less reasonable measure of central tendency than is the arithmetic mean (cf. subjects 4, 5 and 9); the variance in the groups has, therefore, been analysed for significance simply on the basis of deviation from the arithmetic mean. Even so, the value for Z is practically equal to its 1% point, i.e. there is a highly significant association between the individual and his louse counts.

The frequency distribution of natural populations

The distribution of infestation values is shown in Fig. 1. The distribution has been treated in three parts: from 1 to 30 in intervals of 5, from 31 to 150 in 10's, and from 151 to 500 in 50's.

The curves, if plotted with equal horizontal spacing from 1 to 500, would be of inordinate length. They have, therefore, been contracted progressively from left to right, by basing the horizontal spacing on the log of $(x+20)$, x being each successive point on the scale. (A scale based directly on $\log x$ causes an excessive disproportion between the initial and later spacings.) The scale still

Table 1

Subject	Dec.		Jan.		Feb.		Mar.	Apr.		Arithmetic mean	σ	Coefficient of variation	Geometric mean	Mean log $(x+1)$	σ
1	4	8	1	2	4		—	0		3	2·86	95	2·3	0·52	0·338
2	8	5	33	7	0		—	1		9	12·18	135	4·5	0·74	0·536
3	50	53	147	110	62		—	37		77	42·71	55	68·2	1·84	0·224
4	8	38	120	133	139		77	430		135	139	103	57·9	1·77	0·552
5	32		3	1	19		—	75		26	30	115	12·2	1·12	0·654
6	45		14	5	9	18	14	—	7		16	13·56	85	12·8	1·14	0·292
7	66		370	131		68		—	7	41	114	131	115	63·6	1·81	0·555
8	51		17	41		5		—	45		32	19·78	62	24·7	1·41	0·394
9	303		140	34		0		270	121		144	122	84	59·3	1·78	0·935

represents an arithmetic progression of values, but the curves are, as it were, observed in perspective.

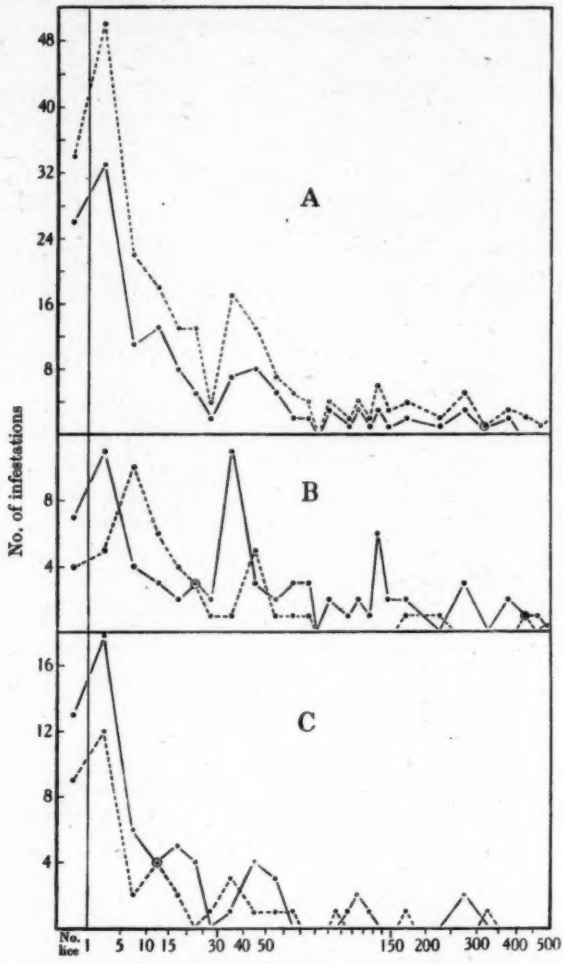

Fig. 1. The frequency distribution of infestations. A. From all sources: whole line, original counts; interrupted line, initial counts. B. Comparison of initial counts of two common lodging houses. C. Comparison of original counts of two Salvation Army hostels.

The frequency curve might be regarded as unimodal from inspection of the general curve (A) (cf. Peacock (1916) for body lice, and Buxton (1938) for head lice), but if the results from each community are treated separately, (B and C), at least two modes in the distribution are revealed. These do not necessarily coincide for different communities (cf. the two lodging houses examined (B),

and the two Salvation Army hostels (C)), so that the troughs may be obliterated when records from different sources are combined.

In the present data, the frequency curve has the first peak between 1 and 10, the second between 30 and 60, and possibly other peaks at about 150 and higher. For convenience of handling, although the data for high infestations are scanty, the curve will be here regarded as having a third peak between 100 and 150. Hence the infestations have been divided into four groups, viz. infestations of 1–25, 26–80, 81–200, and over 200, each of which is regarded as corresponding to a mode.

Possible factors affecting the population level

The counts given in Table 1 were examined from the point of view of seasonal variation. A consistent tendency to decrease can be shown on treatment of the data, but is statistically insignificant.

In Table 2 the average infestation per man is given for men wearing different combinations of under-garments. One or two counts (e.g. one count of 2600 and one of 1217) were so high as materially to affect the averages; the geometric means of the groups are, therefore, also given. The infestation levels, judged by either measure, are independent of the number of under-garments worn. This conclusion is further supported by the distribution of the negative cases. Thus, if the number of under-garments affected the probability of a low infestation, the negatives would reasonably be expected to be most numerous in either the 'shirt only' group or the three garment group; actually they are relatively most numerous in an intermediate group (13 out of 57 examined).

The elements of a louse population

Adult-larval ratio

The adult-larval ratio for 16,527 lice examined is 1 : 3·6. Table 3 shows the relationship between population density and the adult-larval ratio. The densities are taken as those for the particular garment aspects in question, and not those for the subjects. The population values given are the totals for all garment aspects having populations within the appropriate limits.

Taking the total of all garments (columns 8–11): at densities of 1–3 the population on the inner aspects consists of approximately equal numbers of adults and larvae; the ratio increases as the density increases up to about 10 lice per garment aspect, after which it levels out at a range of 1 : 4 to 1 : 6, except for an occasional aberrant group. The higher larval ratios tend to occur at higher densities. If the ratios for shirts worn against the skin are considered, this relative increase of larvae at high densities becomes more apparent; from a range of between 1 : 4½ and 1 : 5½, the ratio suddenly increases, at densities of 563–1000 to 1 : 9½. Evidence is given later that at densities of over 1000 there may be a reversal of the ratio, due to exceptional conditions.

Table 2

	Shirt only	Shirt + one extra garment	Shirt + undervest	Shirt + undervest + one extra garment	Miscellaneous combinations of garments
No. of cases	119	22	44	14	6
Total lice	9672	1961	3690	895	323
Average	81	88	84	64	54
Geometric mean	10·72	23·99	10·72	27·54	13·18
Distribution of negative cases	17	1	13	3	—

Table 3

Population of garment aspect	Shirt against skin		Undervests		Second garment		Totals		Ratios	
	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
1	5/7	10/14	6/2	7/7	5/7	6/4	16/16	23/25	1 : 1	1 : 1·1
2–3	9/12	13/31	10/7	16/17	12/15	12/17	31/34	41/65	1 : 1·1	1 : 1·6
4–6	27/48	28/47	13/24	18/34	14/19	15/12	54/91	61/93	1 : 1·7	1 : 1·5
7–10	20/59	42/105	3/30	4/10	3/30	3/7	26/119	49/122	1 : 4·6	1 : 2·5
11–18	37/204	58/117	19/125	21/50	8/33	—	64/362	79/167	1 : 5·7	1 : 2·1
19–32	79/414	38/94	34/120	25/51	21/76	7/21	134/610	70/166	1 : 4·6	1 : 2·4
33–57	104/481	98/235	35/168	4/35	—	—	139/649	102/270	1 : 4·7	1 : 2·7
58–100	149/451	34/128	8/90	—	—	—	157/541	34/128	1 : 3·5	1 : 3·8
101–178	205/1417	197/345	59/349	—	—	—	264/1766	197/345	1 : 6·7	1 : 1·8
179–316	236/1280	—	23/191	75/110	—	—	259/1471	75/110	1 : 5·1	1 : 1·5
317–562	309/1635	—	—	—	—	—	309/1635	—	1 : 5·3	—
563–1000	146/1378	—	155/600	—	—	—	301/1978	—	1 : 6·6	—
over 1000	206/844	—	—	—	480/645	205/230	—	—	—	—
Totals	1532/8230	518/1116	365/1706	170/314	543/825	248/291	—	—	—	—
Ratios	1 : 5·36	1 : 2·16	1 : 4·67	1 : 1·85	1 : 1·52	1 : 1·17	—	—	—	—

The highest larval ratio is found on the inner aspect of shirts worn against the skin (bottom row of Table 3). The rather lower ratio for undervests is probably due to many of these being of the sleeveless type, with few seams. On all garments the 'total' ratio on the outer aspect is approximately 1 : 1–1 : 2.

The ratios in the successive territory areas (obtained by adding columns 5 and 6 of Table 3) show the relative preponderance of adults in the outer two territory areas:

	Area 1	Area 2	Area 3
Totals	365/1706	713/1139	248/291
Ratios	1 : 4·7	1 : 1·6	1 : 1·17

Sex ratio

The sex ratio of 1786 adults sexed is 1 : 1·27 (males to females). On the successive aspects of two under-garments (excluding sleeveless vests and cases where two shirts were worn), the males are predominant on all aspects but the innermost:

	Males/females (inside + outside)	Ratios
Shirts against skin	505/746 + 226/205	1 : 1·48 + 1·1 : 1
Sleeved undervests	119/143 + 74/61	1 : 1·2 + 1·2 : 1
Shirts over sleeved undervests	29/16 + 19/15	1·9 : 1 + 1·3 : 1

Detailed examination of the data failed to reveal a shift of the sex ratio with increasing density, such as described by Buxton (1937) for exceptionally high densities of head lice.

The distribution of a louse population on the garment

Shirts worn against the skin

Table 4 summarizes the regional distribution of lice on 133 shirts worn against the skin. The habitat areas referred to are as follows:

Neck band (*Nb*).

Breast (*B*), areas of reinforced cloth where the shirt is buttoned in front, including the seams.

Shoulders (*Sh*), front and back seams of epaulettes, seam between the shoulder blades, and seam at head of sleeve except the 'armpit' area, including the area between these seams.

Armpits (*Ap*), the four-rayed star formed by the seams in the armpit, the area extending for approximately 1–1½ in. along each seam.

Side seams (*SS*), from the armpits to the bottom of the shirt.

General surface (*GS*); this is divided into top and bottom of the front (*Ft.Fb.*), and back (*Bt.Bb.*), by a line drawn at the level of the base of the breast area, and a corresponding line on the back surface.

Sleeve seams (*Sl.s*).

General surface of sleeves (*Sl.g.s.*), including cuffs and their seams.

On the inner aspect females are relatively most numerous in the armpits, which is a favourite oviposition area, and are also markedly preponderant (ratio 2 : 1) in the side seams. On the 'general surface' males were found to be predominant on the lower front portion, but not elsewhere. This difference was consistent for all densities below 300.

Table 4

Table 4

Habitat	Total sexed (males/females/larvae) Inside	Outside	Sex ratio		Total sexed and unsexed (adults/larvae) Inside	Outside	Age ratio		Percentage distribution Inside	Outside
Nb	6/14/111	2/1/20	1 : 2½	2 : 1						
B	30/37/397	19/17/80	1 : 1½	1 : 1						
Combined	36/51/508	21/18/100	1 : 1½	1 : 1	108/706	49/132	1 : 6½	1 : 2⅔	8	13
Sh: Right	31/58/742	7/5/28			113/897	15/28				
Left	51/75/897	9/6/33			156/1019	21/33				
Total	82/133/1639	16/11/61	1 : 1⅔	1 : 1½	269/1916	36/61	1 : 7	1 : 1⅔	23	7
SS: Right	30/56/731	6/8/54			136/996	17/58				
Left	22/46/666	6/12/44			97/861	23/49				
Total	52/102/1397	12/20/98	1 : 2	1 : 1⅔	233/1857	40/107	1 : 8	1 : 2⅔	22	10
Ap: Right	9/26/231	2/5/11			43/276	8/11				
Left	7/25/324	4/3/21			52/448	11/34				
Total	16/51/555	6/8/32	1 : 3½	1 : 1½	95/724	19/45	1 : 7⅔	1 : 2½	8	4
Sl.s.: Right	22/27/255	6/4/9			58/320	12/13				
Left	20/44/298	5/2/15			77/357	12/23				
Total	42/71/553	11/6/24	1 : 1¾	2 : 1	135/677	24/36	1 : 5	1 : 1½	8	4
Sl.gs.: Right	14/26/145	8/6/34			68/200	18/42				
Left	20/23/173	11/18/36			60/213	34/42				
Total	34/49/318	19/24/70	1 : 1½	1 : 1½	128/413	52/84	1 : 3½	1 : 1⅔	6	9
GS: *Ft.*	22/34/533	11/9/82					1 : 9½	1 : 2⅔		
Fb.	54/49/272	31/24/103					1 : 2⅔	1 : 2		
Bt.	34/45/387	16/10/48					1 : 5	1 : 2		
Bb.	31/45/384	23/27/122					1 : 5	1 : 2½		
Unspecified	2/2/5	2/0/0								
Total, *GS*	143/175/1581	83/70/355	1 : 1½	1 : 1	489/1920	282/481	1 : 4	1 : 1⅔	25	53

Table 5

	Inner surface Adults	Larvae	Age ratio	Total	Outer surface Adults	Larvae	Age ratio	Total	Whole garment Adults	Larvae	Total
Seam areas	840	5880	1 : 7	6720	168	381	1 : 2½	549	1008	6261	7269
General surface areas	613	2328	1 : 3¾	2941	332	565	1 : 1⅔	897	945	2893	3838
Summation	1453	8208	—	9661	900	946	—	1446	1953	9154	11107
Seams as percentage of total	58	72	—	70	34	40	—	38	51	68	65

The proportion of lice on the inner and outer aspects, with different total densities of population, is as follows (expressed as the ratio outside/inside):

Density	1–3	4–10	11–18	19–32	33–57	58–100	101–178	179–316	317–562	Over 562	Total
Ratio	1 : 2	1 : 8	1 : 6	1 : 7	1 : 5½	1 : 4½	1 : 7	1 : 6½	1 : 7½	1 : 7½	1 : 6½

Thus there is no relative increase of lice on the outer aspect with increasing density. The population of an infested shirt tends to be distributed as follows (last columns of Table 4):

Inside: approximately a quarter of the total on each of the three areas—shoulder seams, side seams, general surface, with the remaining quarter divided more or less evenly between the remaining areas.

Outside: half of the population on the general surface, the other half distributed more or less evenly over the remaining areas.

Thus the proportions of the population on the seam areas and general surface areas differ for the two aspects of the shirt. These two main classes of habitat differ also, even for the same aspect, in the age distribution of the lice (Table 5). By seam areas are meant side seams, armpits, sleeve seams, neckband, breast and shoulders. 'General surface' areas include the main general surface and the general surface of the sleeve.

The analysis shows that approximately two-thirds of the total shirt population is in the seam areas; this general figure, however, bears little relation to the actual distribution when each shirt aspect and age group is considered separately.

With regard to the four main parts of the general surface (Table 4), the age distribution is more or less normal on both halves of the inner back surface but varies on the inner front surface from a very high larval proportion on the top half to a very high adult proportion on the lower half. As shown earlier, an unusually high proportion of these adults are males.

The effect of population density on the distribution in different habitats is illustrated in Fig. 2. The distribution curves may be divided into three phases, which correspond with the main phases of the frequency curve of distribution (Fig. 1), the first phase to the first mode, the second to the second and third modes, while the third corresponds to extreme densities (200 and over). In phase 1 the 'general surface' population is proportionately high at first (compare day 1 of Fig. 6). This suggests that many of the 1–10 density cases are instances of commencing reinfestation, where the shirt population is not in balance with the 'clothes-reservoir' population. In phase 2, the average values given in Table 4 are fairly consistently held, but in phase 3 the relative distribution is again upset.

Distribution on successive surfaces of two under-garments

Table 6 gives the distribution on the successive aspects, from within outwards (inside + outside of undervest, inside + outside of shirt) of all cases where a sleeved undervest and shirt were worn. The cases have been grouped according to the total population of the two garments. The lower part of the table gives

the counts expressed in relation to a standard count of 100 on the inner aspect of the inner garment.

Over all densities the approximate distribution is: half of the lice are on the inner territory area, most of the remainder in the second territory area, and only a relatively small number on the outside of the outer shirt.

The age ratios are, in approximate figures, 1 : 4, 1 : 2½, 1 : 1½. In the individual groups the ratios, at least for the first and second territory areas, agree fairly well with this average at densities over 10, except for one aberrant ratio. At densities from 1 to 10, however, there is a relative preponderance of adults. This again suggests that such cases are often in the early stages of reinfestation, i.e. that many subjects have low counts because of a recent change of under-clothes, rather than because of their potential level being below 10.

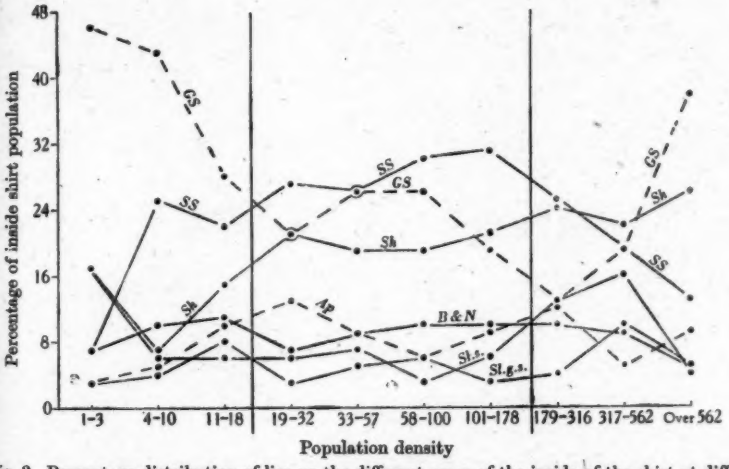

Fig. 2. Percentage distribution of lice on the different areas of the inside of the shirt at different densities. The three phases in the relation of the curves to each other are indicated by upright lines. In phase 2 (normal infestations) approximately one-quarter of the population inhabits each of three principal areas; at lower densities the relation is upset, indicating that many such populations are not in equilibrium.

Distribution on pants

Of the subjects examined who were wearing pants, in only 16 cases were the observations made sufficiently detailed to be of value. Of these, in 8 cases, a shirt, undervest and pants were worn, and in 8 cases, a shirt and pants. The comparative infestations, expressed as the totals inside + outside, were:

	Pants	Vest	Shirt
Adults/larvae	31/55 + 10/12	14/86 + 20/14	16/49 + 10/11
Adults/larvae	50/33 + 9/0	—	44/152 + 12/33

The summed totals for the 16 pairs of pants are 81/88 + 19/12, age ratios inside and outside of 1 : 1 and 1½ : 1, i.e. the pants appear to be comparable, in respect of age distribution, to the outer of two under-garments.

Table 6

Class interval	No. of cases	Adult/larval counts				Total	Counts per territory area			Age ratio for 1st and 2nd territory area		
1–5	11	8/8	+ 7/5	4/7	+ 1/0	34	8/8	5/12	1/0	1 : 1	1 : 2	
6–10	8	8/12	+ 6/11	5/7	+ 8/1	58	8/12	11/18	8/1	1 : 1½	1 : 1½	
11–20	5	6/24	+ 3/14	8/11	+ 3/11	80	6/24	11/25	3/11	1 : 4	1 : 2	
21–50	9	29/154	+12/37	5/33	+ 5/15	290	29/154	17/70	5/15	1 : 5	1 : 4	
Over 50	3	19/99	+23/36	13/43	+ 6/3	242	19/99	36/79	6/3	1 : 5	1 : 2	
Total	36	70/297	+45/103	35/101	+23/30	704	70/297	80/204	23/30	1 : 4¼	1 : 2½	1 : 1½
1–5		100	38	68	6							
6–10		100	83	60	43							
11–20		100	55	63	45							
21–50		100	27	21	11							
Over 50		100	50	47	8							
Total		100	43	37	15							

Table 7

Habitat	Inside	Outside	Ratio inside/outside	Age ratio inside surface
Waistband	14/19	2/1	11 : 1	1 : 1½
Crutch	17/34	6/8	4 : 1	1 : 2
General surface	26/23	4/1	10 : 1	1 : 1
Leg seams	7/10	0/0	4 : 1	1 : 1½
Leg general surfaces	17/2	7/2		8 : 1
Total	81/88	19/12	5½ : 1	1 : 1

Table 7 gives the distribution of adults and larvae in habitat areas. 'Waistband' includes the front opening; 'crutch' includes the central back seam up to the waistband; the 'general surface' excludes the legs.

Two points of interest are brought out in the table. The ratio inside/outside is markedly lower for the crutch than for the waistband and general surface. This suggests that the outside of the crutch is receiving lice from the fork of the trousers, and supports the suggestion of Peacock (1916) that the latter area is a focus of infestation.

On no part of the inner surface is there an age distribution comparable to that found in the breeding areas of the innermost territory area over the torso. The nearest approach to a 'breeding site' ratio is that of 1 : 2 on the inner crutch surface.

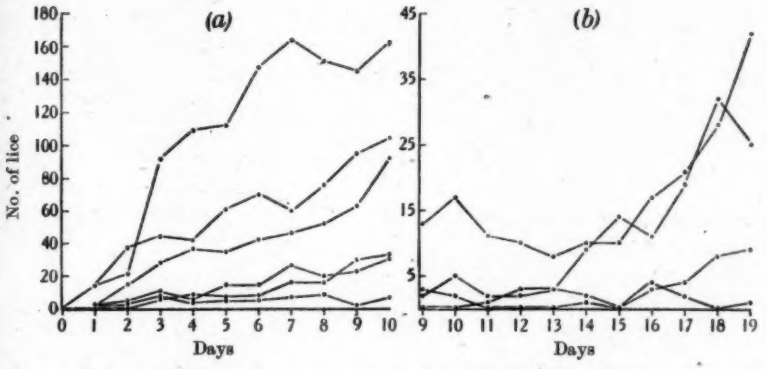

Fig. 3. (*a*) The individual curves for 6 men (group A) for the first 10 days after donning clean shirts, and (*b*) for four men from group C after partial delousement by 8 days' exposure to a pediculicide.

The infestation of clean shirts under verminous conditions

Twenty-one men, whose infestation levels were known from previous observations, were divided into three comparable groups each containing three men believed to belong to mode 1, three to mode 2 or 3, and one to mode 4. Each man was given a new army shirt, and instructed to wear it continuously against his skin. In the case of group B, the shirts were dressed, before issue, with a pediculicide, which continued to destroy practically all lice migrating on to them for the next 5–7 days. The group C shirts were similarly treated and retreated on the 8th and 16th days, so that they remained continuously unfavourable to lice up to at least the 20th day. Details of this work are given in another paper (Craufurd-Benson & MacLeod, MSS.).

The history of infestation of group A indicates what happens when a fairly representative group of men, habituated to a lousy environment, change their under-clothing. The individual curves, with one exception which will be dis-

cussed later, are illustrated in Fig. 3*a*. They show the range of variation resulting from the interplay of the two variables, reservoir pressure of population, and degree of inherent susceptibility, the environment being assumed to be constant.

The infestations in group C, after the 20th day, indicate the effect of the environmental contribution alone, since the clothes reservoirs have been exhausted by the unfavourable period of 20 days. Although some residual effect of the pediculicide will be present from 8 to 12 days after application, this is common to all the men, so that their relative rates of infestation are a measure of possible individual variation in response to a given environment. On the 28th day, 12 days after the last treatment, the infestations were 1, 1, 1, 0, 2, 2, 1, i.e. the rate of invasion was practically uniform.

The group B men, dressed only once, may be regarded as partially deloused men. Observations were continued on four of them for 19 days (Fig. 3*b*). If oviposition became possible by the 8th day, the second generation should begin to appear from the 17th day onwards. The sudden rise of two of the curves about the 16th day is probably due to this cause, i.e. up to the 16th day the infestations were due either to immigration from the clothes or invasion from the outer environment. Their comparative uniformity shows that, where the part played by the clothes reservoir in reinfestation is reduced, relative to that of the environment, the range of variation in individual infestation rates is also reduced.

Insusceptibility

If a man is exposed to the risk of infestation but remains free of lice it is at least possible that he is unattractive to lice, or an unsuitable host. An example is case 80 (subject 1 of group A), who had changed his shirt two days previous to the first examination. His louse count was 1/3+2/0 (inner and outer surfaces of shirt). This low count was attributed to the recent change of clothing, and a week later he was used for experiment. He was given a clean untreated shirt which he wore against his skin, without washing or changing, for 10 days. His daily counts, expressed as adult/larvae inside and outside, were:

Day...	1	2	3	4	5
	2/0+0/0	0/3+0/0	1/7+0/0	1/2+1/1	2/2+1/1
Day...	6	7	8	9	10
	2/4+0/0	2/3+1/1	4/2+1/1	2/0+0/0	6/1+0/0

There is here evidence of continuous infestation, but obvious failure of larvae (cf. 3rd to 4th and 8th to 9th days) or adults (cf. 1st to 2nd and 8th to 9th days) to establish themselves.

Other suggestive evidence of actual insusceptibility was obtained in a few cases, viz.:

Case 47/76. Among a group of men, examined on the same day, in a search for suitable material for experiment, four, all from common lodging house A, gave a history of having changed their under-garments within the previous two days. Their louse counts were all low,

and they were rejected. Three days later these men were re-examined with the following result:

Case no.	1st examination	2nd examination	Increase
46	0/1+0/0	4/4 +0/2	9
47	0/1+1/0	0/2 +0/0	0
49	1/3+0/0	5/15+1/1	18
50	1/3+1/0	5/2 +2/0	4

(Counts expressed as adults/larvae, inside + outside of shirt.)

Case 47 was re-examined 26 days later (case 76). He reported that he had not changed his under-clothing since he was last examined, yet his count was 2/1+0/1.

Case 159. From a low-class Salvation Army hostel. History—shirt not changed for about 10 days previously.

Louse counts: undervest, 0/0+1/4; shirt, 0/0+0/1; i.e. few lice and none on the inner aspects.

Case 125 (Negro). From common lodging house A. History—shirt and undervest worn about a week (the vest was fleecy-lined and peculiarly suitable for lice). The count was: vest, 0/0+1/0; shirt, 1/5+3/7; i.e. greater numbers on the outer than on the inner aspects —a reversal of the usual order.

Apart from these four cases, no evidence of insusceptibility was obtained and it is believed that the phenomenon is uncommon. Hase (1915) includes insusceptibility in his four categories of individual reaction to infestation, but does not state the probability of its occurrence.

The development of an infestation

The development of infestation on the shirts of six of the seven men in group A is shown in Fig. 4*a*.

The total adult/larval counts for each day, are:

Day...	1	2	3	4	5
Total	21/10	26/56	41/148	40/168	42/194
Ratio	2 : 1	1 : 2	1 : 3·5	1 : 4	1 : 5
Day...	6	7	8	9	10
Total	56/235	64/257	62/262	100/258	130/299
Ratio	1 : 4·5	1 : 4	1 : 4	1 : 2·5	1 : 2

Thus, after 3 days of rapid increase, a primary balance is established between the outer clothing and the shirt; the numbers, particularly of larvae, then increase more slowly up to the 9th day. The adult/larval ratio also remains fairly constant from the 4th to the 8th day. On the 9th it is upset, probably by commencement of moulting of those larvae which had first migrated to the shirt, as recently hatched larvae, 7 or 8 days before. The rapid increase of adults is maintained on the 10th day, but the ratio is not correspondingly altered because a compensating increase of larvae has now commenced, resulting from the eggs of the earliest colonist females. The first of a 'native' population born on the garment in question, has now begun to appear.

Fig. 4*b* depicts the daily infestation of one subject from the group (case 81), who was known to have a low reservoir population, three counts over the previous month having given values of 14, 0 and 3. The infestation, up to the

9th day, is composed of relatively more adults than is the average for the group infestation, and illustrates the increased significance of the 'invasion increment' in building a shirt infestation, where the clothes reservoir is of low density.

Both graphs demonstrate the ebb and flow of lice from one aspect of the shirt to the other, obscuring the consistency of the increase in infestation.

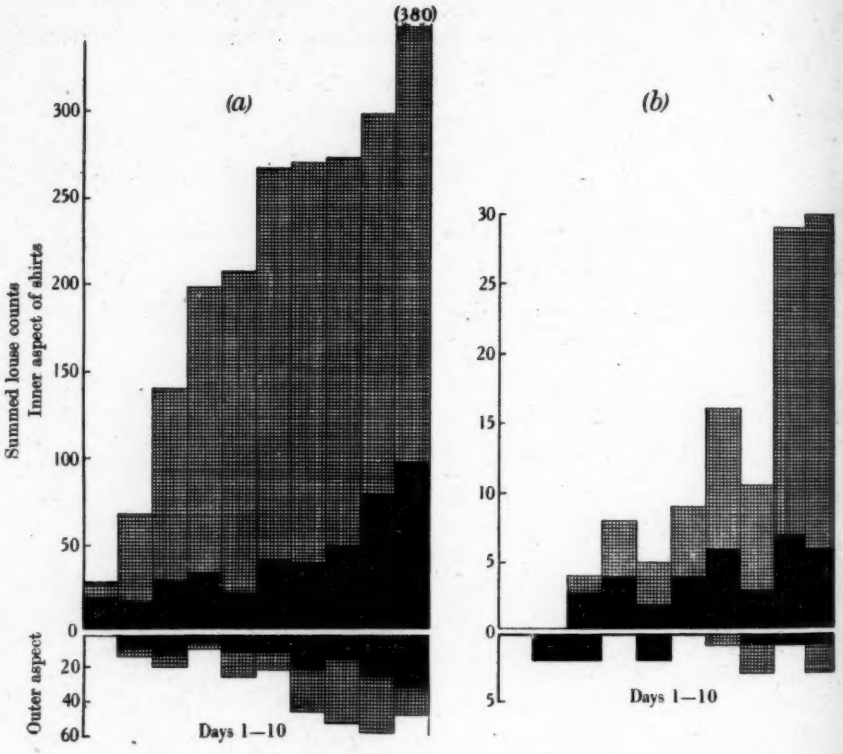

Fig. 4. (*a*) The daily total infestation of the shirts of six men from group A. The lower part of the diagram refers to the outside of the shirt. Black shading equals adults, interrupted shading, larvae. (*b*) The daily infestation for one of the six men, known to be lightly infested. Note the relative preponderance of adults, until the appearance of second generation lice on the 9th day.

Colonization of different habitat areas

Fig. 5 illustrates the progressive colonization of the different areas of the shirt, based on the total louse counts for the six men in group A. The infestation for the first 2 days, and each subsequent alternate day, is illustrated; the daily totals are given in Table 8.

The figure illustrates the following general trends in the process of colonization:

Day 1. Colonists mostly adults. General surface invaded.

Day 2. Migration to seams, larvae congregating there, and on shoulder areas. Adults present on outer aspect, probably migrating inwards.

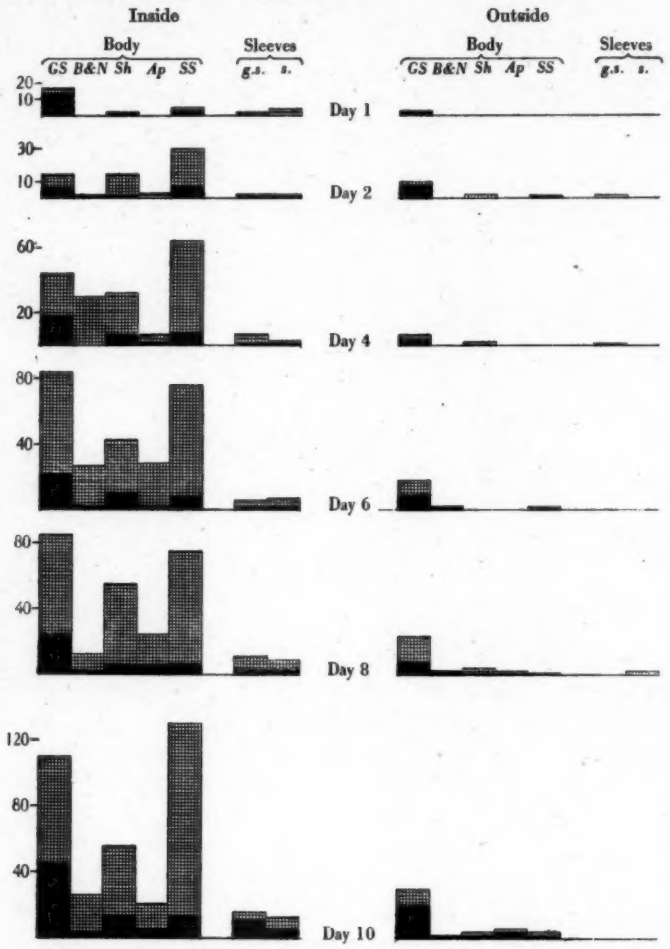

Fig. 5. The development of infestation of clean shirts, according to areas. The first 2 days, and each subsequent alternate day, has been figured. Black shading represents adults, interrupted shading, larvae.

Day 4. Larvae in the seams markedly more numerous. More numerous on the breast, neck and shoulder regions than on the general surface, suggesting that the path of migration to the inside is from above rather than from the trousers. General surface still principally populated by adults, who have presumably not yet reached the egg-laying sites—the seams.

Table 8. *Louse counts expressed as males/females/larvae.*

	GS		*B+Nb*		*Sh*		*Ap*		*SS*		*Sl.gs.*		*Sl.s.*		Total	
Day	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
1	7/8/2	0/1/1	—	—	0/1/0	—	—	—	0/3/2	—	0/1/1	—	0/0/4	—	7/13/9	+ 0/1/1
2	6/1/8	6/2/2	0/0/1	—	0/1/14	0/0/1	0/0/3	—	2/5/23	1/0/0	0/1/1	0/0/2	0/1/1	—	8/9/51	+ 7/2/5
3	7/6/15	5/4/3	0/0/19	0/0/1	2/4/28	1/1/2	1/0/6	—	1/5/71	0/0/1	1/0/0	0/2/0	1/0/2	—	13/15/141	+ 6/7/7
4	13/4/27	2/1/4	1/0/29	—	2/5/25	0/1/1	0/0/7	—	1/7/67	—	0/1/6	1/0/0	0/1/2	—	17/18/163	+ 3/2/5
5	6/6/39	5/5/7	0/0/25	0/1/0	2/2/25	—	0/0/21	1/0/0	4/4/67	0/1/1	1/0/2	2/2/1	0/0/6	—	13/12/185	+ 8/9/9
6	13/9/61	5/4/9	1/1/25	0/0/2	4/6/33	—	1/0/27	—	3/4/68	0/2/0	2/1/3	—	0/0/7	—	24/21/224	+ 5/6/11
7	12/8/96	10/5/17	0/3/12	0/0/2	4/6/45	0/0/3	1/0/15	1/1/2	0/6/57	1/3/0	0/1/2	0/0/1	0/1/5	—	17/25/232	+12/9/25
8	15/9/61	3/6/14	2/0/10	1/1/0	0/6/49	1/0/3	1/4/19	0/1/1	3/3/70	0/0/1	3/0/8	—	0/3/6	0/0/2	24/25/223	+ 5/8/21
9	18/19/58	7/12/26	0/3/18	1/0/1	10/6/55	0/1/0	3/2/16	0/1/1	4/3/57	1/0/7	2/0/8	0/0/1	2/5/10	—	39/38/222	+ 9/14/36
10	27/18/65	14/7/9	2/2/22	1/0/0	6/8/42	1/0/4	1/5/12	3/2/1	2/12/130	2/1/2	4/8/4	—	4/1/8	—	46/54/283	+21/10/16

Day 6. Population of armpits and shoulders increased. An increase of the general surface population, principally of larvae; this is reflected in an increase of the numbers of lice on the outside general surface, probably an 'overflow' effect.

Day 8. A slight increase on the shoulders and sleeves, but no marked change from the 6th, or indeed from the 5th day position (Table 8).

Day 10. Advent of 'native' lice. The seam concentrations of larvae have increased by hatching, and there has been generally an explosive increase of adults by moulting, resulting in an 'overflow' increase of adults on the outer surface.

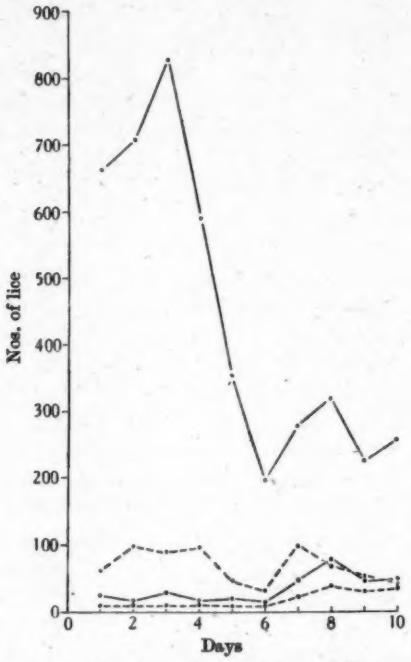

Fig. 6. The daily counts for the shirt of one excessively lousy subject in group A. The whole lines indicate the count on the inner surface, the interrupted lines that on the outer surface. The upper line of each pair refers to larvae, the lower to adults.

Observations on exceptionally heavy infestations

The seventh man of group A (case 86) was an exceptionally verminous subject. In one day his shirt count rose to over 700, and was almost 1000 by the 3rd day. This heavy infestation consisted of an abnormally high proportion of larvae, mostly 1st and 2nd instar, on the inner shirt surface. After a catastrophic decrease (Fig. 6) the population again steadied at a new level. Corresponding to this change, the adult/larval ratios on the two aspects changed markedly, the outer ratio approximating to the normal of 1 : 1 or 1 : 2, while the inner steadied at an unusually low larval proportion of 1 : 3–1 : 3½, as compared with the normal ratio of about 1 : 5.

Two infestations exceeding 1000 were recorded. The ratios in these were:

Case 74. Sleeveless vest and shirt. Total count 2600. Adult/larval ratios: undervest, 1 : 4½, 1 : 1½; shirt, 1 : 1⅓, 1 : 1.

Case 114. Two shirts and no vest. Total count 1217. Ratios: inner shirt, 1 : 4, 1½ : 1; outer, 2 : 1, 8 : 1.

In the second case the larval ratio is unmistakably abnormal, with a marked adult preponderance on all surfaces but the innermost, and it is at least possible that this population had recently suffered a severe reduction of its larvae, similar to that observed in case 86 (Fig. 6).

Three other instances of relatively high infestation (500–1000) were obtained. In all three the subjects wore a shirt against the skin. Case 257, with a total population on the shirt of 866, showed inside and outside ratios of 1 : 74 and 1 : 9 (compare days 1–3, Fig. 6). Case 186, shirt population 862, showed the approximately normal ratios of 1 : 5 and 1 : 2½. Case 29, shirt population 518, showed ratios of 1 : 2½ and 1 : 1.

These figures might be interpreted as indicating that the infestation in case 257 was building up to an unbalanced climax preceding a collapse, that of case 29 had just 'crashed' through disappearance of larvae, while case 186 apparently still possessed a balanced, or stable, population.

At lower densities sudden changes of the level of a shirt population have been observed, but these could usually be related to some physical factor. Thus, in men under regular observation it was noticed that if on arrival their shirts were damp with perspiration, the damp areas were invariably deserted by the lice, the total count being sometimes much reduced. Such fluctuations were usually redressed by the following day. With senile subjects, where the lower part of the shirt was often soiled with urine, the lice were similarly found to forsake areas such as the lower side seams, when these were wet, but the armpit and shoulder counts in such cases were often correspondingly increased.

Résumé

From a study of natural infestations of over 200 sets of under-garments, the following conclusions seem permissible, at least as provisional working hypotheses.

Under-garment infestations tend to fall into four groups: those with totals of under 10 lice, between 30 and 60, 100 and 150, and those of over 200 lice.

Individual subjects tend to remain in a given infestation group.

The number of under-garments worn by a subject does not influence his infestation level.

Except at extremes, under-garment populations show certain characteristics which allow the 'normality' of the population to be judged. (An infestation is held to be 'normal' when the proportion of lice on the under-garments to those on the outer clothing is in equilibrium, so that alteration of the numbers on, say, the shirt occurs only as a result of alteration of the subject's total infestation.) The 'normal' characteristics are: (*a*) the relative distribution of the population in the different areas of a garment (see next paragraph), (*b*) the proportion of the population on the inner and outer aspects, this ratio remaining fairly constant irre-

spective of density, and (*c*) the proportion of adults to larvae on the different aspects, which is fairly constant for densities between 10 and 500.

The habitat distribution on the inner surface for 'mode 2' and 'mode 3' infestations is constant. Modes 1 and 4 infestations show irregularities, but their normality may, however, be judged by the age ratio and the distribution between the different aspects or territory areas.

An under-garment population of low density because of a recent change of under-garments will naturally not be in balance with the sources from which it arises; it is recognizable because of (*a*) the abnormal habitat distribution, too high a proportion being on the general surface, (*b*) the abnormally high adult proportion, approximating to 1 : 1 or 1 : 2, and (*c*) the abnormal distribution between the inner and outer aspect, or between the first two territory areas, relatively too many lice being in the outer territory. At very high levels of density, the population may be unbalanced because of violent fluctuations from unknown cause, expressed principally by alteration of the larval population level. Such a population is recognisable before or after a decimating fluctuation by the excessively high or excessively low larval proportion respectively.

From a study of the process of colonization of clean shirts issued to infested subjects, the following points emerged.

The shirt infestation appeared to come into equilibrium with the total population in 3–6 days, after which the population rose slowly until the 9th day, when 'native' lice began to appear, and the primary infestation equilibrium was upset.

The rate of infestation and primary equilibrium level differed markedly from man to man, although they all lived in the same common lodging house. When a similar group was deloused so that the rate of infestation of the shirt was due solely to the invasion increment, little variation was evident in their individual infestation rates. In insusceptible cases, lice failed to establish, so that the subject's infestation at any given time represented little more than the invasion increment for that moment.

During the first day or two after donning of a clean shirt, both the relative distribution in different habitats and the age ratio were abnormal. Similar aberrations were found in natural infestations with a population density below 10, and it is probable that many of these are instances of active reinfestation.

Discussion

The population of the average cheap hostel is by no means predominantly vagrant in character, all grades of working-class men being represented. The observations made should not, therefore, be regarded as a study of lousiness among a class who accept lousiness as natural, but as an indication of what happens when men of all classes are grouped together under conditions which allow of vermin surviving, as for instance in refugee camps or in an army on active service.

In a verminous environment freedom from casual infestation by contact or otherwise, is ordinarily impossible. On the other hand, a verminous environment is obviously not essential to the continuance of an infestation. As a third generalization it may be stated that, in a body of men living in the same environment, wide variations occur in the degree of infestation. The respective parts played by the environment and the man in developing and maintaining an infestation thus invite consideration, and some information has been

obtained on this subject from a study of the infestation of clean shirts worn by deloused, partially deloused and lousy men.

It has been shown that the rate of reinfestation of clean garments is predominantly influenced by the immigration rate from the outer clothing, which varies with the pressure of the population reservoir there. When this source of reinfestation is removed, there is seen to be a basal invasion rate from outside sources, which is fairly uniform over a given group, its value depending on the environment and not on the individual.

In a group of men newly introduced to a verminous environment, the initial infestation should therefore be fairly equal. The fate of the invading lice will, except in cases of insusceptibility, depend on the degree of personal care exercised by each man, so that, after a period, 'outer clothes populations' of varying density are built up in the same group. The chief factor influencing density will usually be the frequency of change of under-garments.

Once the 'clothes reservoirs' are established, the immigration rate on to clean under-garments will vary widely within the group, men with a high clothes reservoir being penalized by more rapid reinfestation. Differences are thus accentuated, so that after a time it should be possible to divide a group exposed to a uniform environment into subgroups of different degrees of lousiness according to the frequency with which they change their under-garments.

Now, the intervals between changes of under-garments are generally divisible into four or more categories: daily or two daily, with men of clean habits, weekly in a very large proportion of men, each alternate week among some men, many of whom have the habit of changing only the outer of two under-garments weekly, and at prolonged and irregular intervals, in careless or very destitute sections of a population. The frequency distribution of infestation tends similarly to show grouping about different density levels, and it is suggested that the two are causally related.

The position may be summed up by saying that infested individuals very soon become victims of their own habits, rather than of their environment, in so far as their louse burdens are concerned. This is well illustrated in Fig. 3*a* (group A men). Although the men in question were exposed to a more or less uniform environment (excluding their beds, which can here be classed with their outer clothing), the shirt infestation levelled out at an equilibrium characteristic of each man. In one respect, however, these men were divorced from their normal routine—they were all obliged to wear the same shirts, unchanged, for the duration of the experiment. Except in the case of one insusceptible man, the light and medium infestations began to increase as a result of the appearance of native lice, indicating that the control of louse populations at different levels is due, not to peculiarities inherent in the subjects, but to their habits, and is upset where the habits are changed.

In control experiments with naturally infested subjects, the post-treatment counts must obviously be regarded in relation to those before treatment. It is essential, to avoid misinterpretation, that the pretreatment counts be representative

of the men's 'normal' infestations. A man chosen at random may have a high potential shirt infestation, but may have changed his shirt, say, the day before examination; his count will be fictitiously low, and treatment appear unduly ineffective. It is suggested that, by the criteria of normality developed above, this source of error may be obviated, if age—and regional—distribution are noted at the first count.

Acknowledgements. The above work forms part of a general study of louse infestation in relation to control measures. We wish to record our appreciation of the encouragement and interest which we met with from Prof. P. A. Buxton, our great indebtedness to Dr W. E. Parry, to whose active assistance and intimate knowledge of the district of London concerned much of the credit for successful completion of the work is due, and finally, our humble gratitude to those many men, who must here be nameless, for the readiness with which they, who owe little to mankind, submitted to experiment to improve the lot of more fortunate people.

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ON *MULTICOTYLE PURVISI*, N.G., N.SP., AN ASPIDOGASTRID TREMATODE FROM THE RIVER TURTLE, *SIEBENROCKIELLA CRASSICOLLIS*, IN MALAYA

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(With 1 Figure in the Text)

The aspidogastrid trematodes form a well-defined group of at most eight genera with a wide distribution. They are known from five continents and they occur in various cold-blooded hosts from both marine and fresh-water habitats, notably, Gasteropod and Lamellibranch molluscs, the larger Crustacea, some Fishes, and Chelonians. The recording of a new genus in a fresh locality is thus of some zoological importance.

Since 1827, when Baer discovered *Aspidogaster conchicola* in *Anodonta*, many attempts have been made to find a satisfactory niche in the scheme of classification of the Trematoda for this interesting group. Burmeister (1856) first commented on the peculiar nature of the adhesive apparatus, which is usually but not invariably a ventral disk bearing a number of suckerlets or alveoli, and separated Aspidobothrii (aspidogastrids) from Malacobothrii (distomes and holostomes) on the one hand, and Pectobothrii (polystomes) on the other. Systematists continued to classify aspidogastrids with polystomes, however, till Monticelli (1892) renamed the three suborders of Burmeister's scheme, Aspidocotylea, Malacocotylea and Heterocotylea.

Poche (1926) placed aspidogastrids in the Digenea, referring them to the suborder Prosostomata and the tribe Aspidogastroidea. All known forms belong to the single family, *Aspidogastridae*. Faust & Tang (1936) raised objections to this scheme, however, and reminded zoologists that the aspidogastrid trematode combines the characters of both *Monogenea* and *Digenea*. It is disqualified for admission to the *Monogenea* by the nature of its adhesive apparatus, which lacks such accessories as chitinous supports, hooklets or anchors, by the posterior position of the excretory pore or pores, and by the simple, rhabdocoelan intestine. On the other hand, the absence of an alternation of generations in the life history militates against its inclusion in the *Digenea*. The simplest type of life history, e.g. that of *Aspidogaster conchicola*, is one in which the sole host is a Gasteropod or Lamellibranch mollusc.

For such reasons as these, Faust & Tang proposed the erection of a new subclass, the *Aspidogastrea*, which ranks equal with the *Monogenea* and the *Digenea*. If their suggestion is acceded, which is by way of being a reversion to the older system of classification, the single order Aspidogastrata Faust, 1923 will stand in place of the family *Aspidogastridae* Poche, 1907.

The following notes on the general anatomy of what is clearly a new genus of aspidogastrid are based on a study of whole mounts of two specimens which were collected from the river turtle, *Siebenrockiella crassicollis*, in Malaya (*Alor Star:* 25 July 1932), by Mr G. B. Purvis, F.R.C.V.S., while he served as District Government Veterinary Surgeon. The specimens are well preserved and they made satisfactory stained mounts, but they do not lend themselves to a full description of the anatomy. Essential characters which support the writer's claim for the erection of a new genus are clearly discernible, however, and a full description of the structure must necessarily await the subsequent collection of other specimens, which will be readily recognizable from the account given in this paper.

Multicotyle purvisi n.g., n.sp.

The body is elongate oval in outline but the anterior end is somewhat attenuated (Fig. 1). The integument lacks spines. Most of the ventral surface of the body is covered by the well-developed adhesive apparatus, which extends from the level of the pharynx to the rounded posterior extremity of the body. The two specimens available measure 2·7 and 2·8 mm. in length, and 0·7 and 0·8 mm. in maximum breadth respectively. The oval disk of the adhesive apparatus of the two specimens measures $2{\cdot}5 \times 0{\cdot}6$ mm. and $2{\cdot}5 \times 0{\cdot}7$ mm. respectively, i.e. is approximately nine-tenths of the body length and body width.

About 144 suckerlets or alveoli are borne on the disk. They are spaced in four longitudinal rows of 36, and they differ in some particulars from the alveoli of other aspidogastrids. Unlike those of *Aspidogaster*, *Lophotaspis*, *Cotylaspis* and others, which are shallow cups with wide openings, they are compressed antero-posteriorly so that their openings are narrow transverse slits. This is no doubt due to the crowding of many alveoli on a small disk. The alveoli also form regular transverse rows, that is to say, they show an opposite arrangement as in *Aspidogaster conchicola* and not an alternating one as in *Lophotaspis interna*. They vary in size in different parts of the disk and measure about 0·11 mm. in the outer and 0·09 mm. in the inner rows anteriorly, diminishing in size to about 0·06 and 0·04 mm. respectively posteriorly. The central portion of the disk is devoid of papillae, and only a few small marginal papillae (whose number could not be determined with accuracy) occur at the periphery of the disk and at the anterior end only. These are situated in the tissue intervening between adjacent alveoli, exactly as in other aspidogastrids.

The numbers of alveoli present on the disk of the adhesive apparatus in different aspidogastrids do not seem to bear any relation to the size of the body or of the disk. Among genera which possess alveoli arranged in three or in four longitudinal rows, however, those of smallest size (e.g. *Cotylaspis* and *Lissemysia*) possess fewest alveoli. Data tabulated below show that while

Multicotyle purvisi is to be regarded as an aspidogastrid of only small to medium size, it possesses more alveoli than any other species, and many more than are to be found in the majority of aspidogastrids, a fact which is embodied in the proposed generic title.

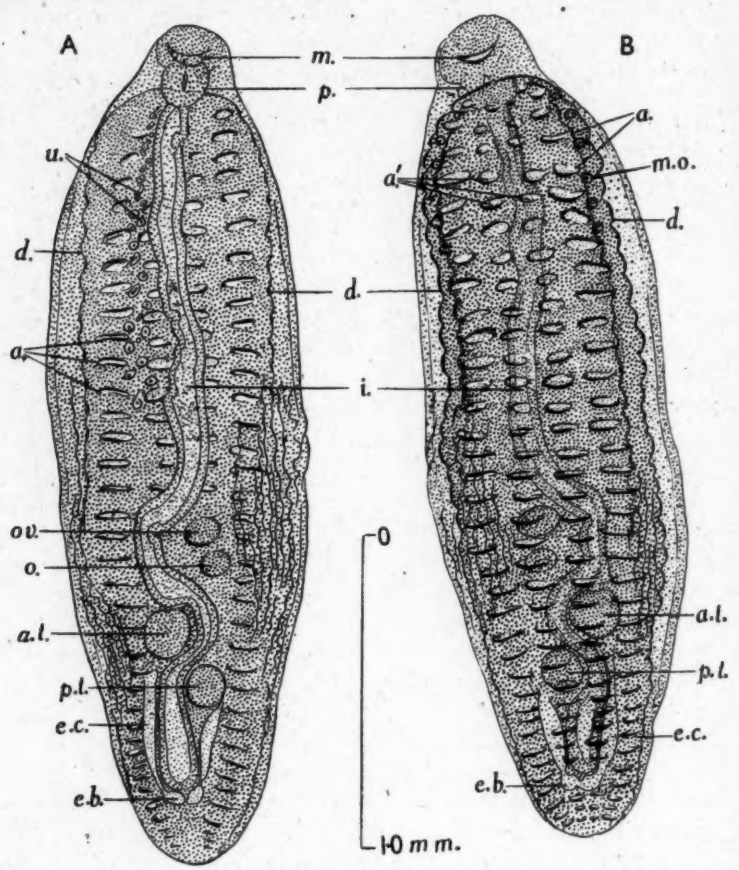

Fig. 1. *Multicotyle purvisi*; entire animal. A, dorsal view. B, ventral view. *a*, alveoli of outer rows; *a'*, alveoli of inner rows; *a.t.*, anterior testis; *d*, disk of adhesive apparatus; *e.b.* excretory bladder; *e.c.* excretory canal; *i.*, intestine; *m.*, mouth; *m.o.*, marginal organ; *o.*, ootype; *ov.*, ovary; *p.*, pharynx; *p.t.*, posterior testis; *u.*, uterus.

The form which most nearly resembles *Multicotyle purvisi* in the arrangement of alveoli on the disk of the adhesive apparatus is *Lophotaspis macdonaldi*, which, like other members of its genus, possesses numerous papillae on the ventral surface of the disk and further differs from *Multicotyle* in having only a single testis (*vide infra*). *Aspidogaster conchicola* is said to possess as many as

Table 1

Species	Length of body (mm.)	Length of disk (mm.)	No. of alveoli	Authority
Aspidogaster conchicola v. Baer, 1826	2·5–3·0	1·75–2·25	64–118	Ward & Hopkins
A. limacoides Diesing, 1834	1·4	1·05	68	Ward & Hopkins
A. decatis Eckmann, 1932	1·3–4·5	0·8*	41	Eckmann
A. enneatis Eckmann, 1932	5·0	0·6*	39	Eckmann
Lobatostoma ringens (Linton, 1907)	2·6	1·6	68–70	Ward & Hopkins
L. kemostoma (MacCallum, 1913)	5·6	1·75	56–60	Ward & Hopkins
Lophotaspis vallei (Stossich, 1899)	9·0	6·0	77	Ward & Hopkins
L. margaritiferae (Shipley & Hornell, 1904)	6·0	?	38	Ward & Hopkins
L. macdonaldi (Monticelli, 1892)	2·5–3·2	0·7*	120	Ward & Hopkins
L. interiora Ward & Hopkins, 1931	3·9	3·34	65	Ward & Hopkins
L. orientalis Faust & Tang, 1936	6·0	3·7	54	Faust & Tang
Cotylaspis insignis Leidy, 1856	?	0·5*	29	Stunkard
C. cokeri Barker & Parsons, 1914	1·5	0·8–0·9	32	Stunkard
C. sinensis Faust & Tang, 1936	1·5	1·0	27	Faust & Tang
Lissemysia indica Sinha, 1935	0·9	?	29	Sinha
Multicotyle purvisi Dawes, 1940	2·75	2·5	144	Present paper

* Body length.

118 alveoli (Stafford, 1896) but this species also has but a single testis. Apart from rare instances such as these, the new genus may be said to possess at least twice the usual complement of alveoli.

The mouth of *Multicotyle purvisi* is a wide crescentic slit transversely set. It is not quite terminal, but is situated about 0·10 mm. behind the anterior tip of the body. An oral sucker such as occurs in *Cotylogaster* is lacking in the new genus, as it is in *Cotylaspis* and *Stichocotyle*. A concentration of muscular tissue is to be seen in whole mounts around the mouth, and this may prove to be the rudiments of a sucker. The presence or absence of an oral sucker does not seem to be of generic importance. Stunkard (1917) regards the lack of such a sucker as one of the characters of the genus *Aspidogaster*, but Eckmann (1932) describes this structure in each of her species *A. decatis* and *A. enneatis*, and states that it is well developed in the latter species.

Immediately posterior to the mouth there is a muscular pharynx, which is circular in outline and measures about 0·15 mm. in diameter. Beyond the pharynx, a simple sac-like intestine extends back in the median plane to within 0·22 mm. of the posterior extremity of the body. It shows certain sinuosities where it passes to the left of the ovary and ootype, to the right of the anterior testis, and to the left of the posterior testis. The intestine is about 0·07 mm. wide, and about one-third of its width is due to the lumen (Fig. 1).

The internal organs cannot be made out with any degree of accuracy, but the gonads are evidently well developed. The ovary is situated about two-thirds of the distance along the body and to the right of the intestine. It is ovoid and measures about $0{\cdot}12 \times 0{\cdot}10$ mm. The ootype occurs immediately behind the ovary and measures $0{\cdot}11 \times 0{\cdot}07$ mm., but appears to be bent upon itself so that it is probably more elongate than these figures indicate. The paired testes, which form one of the most important characters of *Multicotyle*,

are situated in the posterior one-third of the body. They show an oblique arrangement and possess smooth contours. The anterior testis is slightly the larger and measures 0·17 mm. in diameter, as against 0·14 mm. for the posterior testis.

The trematodes were collected and fixed before they had attained to full maturity and no egg capsules are to be seen. The vitellaria are seen to be restricted to the middle and posterior parts of the body, and they are better developed in the latter part. The uterus extends forward to the left of the intestine, and the vas deferens passes to the right of this structure. The excretory bladder is bilobed, and the single excretory pore is situated about 0·15 mm. from the posterior end of the body.

The genera of aspidogastrids have been differentiated by the use of various characters, such as the nature of the adhesive disk and the arrangement of alveoli thereon, the number of testes, the occurrence or lack of a cirrus sac, the presence or absence of papillae on the ventral surface of the disk, the existence of lips bordering the mouth. The following key to the genera is a modification of Eckmann's and includes the genus *Lissemysia* Sinha, 1935, as well as the new genus *Multicotyle*. This key stresses the fact that aspidogastrid genera form a clear-cut series of forms with one, three, or four longitudinal rows of alveoli on the disk of the adhesive apparatus, with underlying series of genera with one or two testes. The new genus presents for the first time the combination of four longitudinal rows of alveoli and paired testes.

Key to genera of aspidogastrids

(1) One row of alveoli. Disk of adhesive apparatus present or lacking. One or two testes.
  (A) Disk present. Suckers confluent. One testis. **Macraspis** Olsson, 1868.
  (B) Disk lacking. Suckers distinct and separate, two testes. **Stichocotyle** Cunningham, 1889.

(2) Several longitudinal rows of alveoli on disk of adhesive apparatus, which is invariably present. One or two testes.
  (A) Three rows of alveoli on disk.
    (*a*) One testis.
      (*aa*) Cirrus sac present. **Cotylaspis** Leidy, 1857.
      (*bb*) Cirrus sac lacking. **Lissemysia** Sinha, 1935.
    (*b*) Two testes. **Cotylogaster** Monticelli, 1892.
  (B) Four rows of alveoli on disk.
    (*a*) One testis.
      (*aa*) Hollow papillae on central region of disk. **Lophotaspis** Looss, 1902.
      (*bb*) Papillae lacking on central region of disk.
        (*aaa*) Mouth with lip-like processes. **Lobatostoma** Eckmann, 1932.
        (*bbb*) Mouth without lip-like processes. **Aspidogaster** Baer, 1827.
    (*b*) Two testes. Alveoli numerous. **Multicotyle** Dawes, 1940.

An additional genus, *Platyaspis* Monticelli, 1892, which is not included in the key but which was listed by Faust & Tang, has been discussed by Stunkard (1917), who explains fully how this genus came to be created, and how resolved to synonymity with *Cotylaspis*.

DIAGNOSIS OF THE GENUS *MULTICOTYLE*, n.g.

Aspidogastrid with the following generic characters. On the ventral side of the body is a disk bearing four longitudinal rows of suckerlets or alveoli, of which the openings are reduced to mere transverse slits. Marginal organs are present at the periphery of the disk only in the anterior region of the body. Papillae are lacking on the ventral surface of the disk elsewhere. The mouth lacks lip-like processes. Two testes.

Type species. *Multicotyle purvisi* n.sp.

Host. River turtle, *Siebenrockiella crassicollis.*

Location in host. Intestine.

Locality. Malaya.

Discovered by Mr G. B. Purvis, F.R.C.V.S., to whom the species is dedicated. Type and paratype deposited in British Museum (Natural History), London.

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NOTES ON SOME NEW SPECIES OF PARASITIC MITES. PART 4

By CHARLES D. RADFORD

(With 8 Figures in the Text)

Introduction

Prof. P. A. Buxton sent me a number of tubes of mite material to examine, and in one bearing the label 'Gerbille, Madras, India, 3. v. 37, C. S. Swaminath', I found a number of mites of the genus *Laelaps*, which were obviously new, in so far as the shape of the anal scutum was decidedly wider than any known species of this genus. This species I have herein described under the name *Laelaps buxtoni*.

Mr G. H. E. Hopkins (Kampala, Uganda) has for some years now been sending large numbers of mites for identification, and it was with pleasure that I examined those in the tube taken from a mole-rat (*Tachyoryctes ruddi*) found at Kapretwa, Mt Elgon, Kenya. The mites are here described under the name *Haemolaelaps tachyoryctes*.

From Miss Isabella Gordon (British Museum Nat. Hist.) I received mites which had been found on a species of bat (*Nyctalus noctula* Schreb.). In addition to a number of the large bat-mites (*Spinturnix acuminatus* Koch) there were a number of *Liponyssus* which form a new species, to which I have given the name *britannicus*.

Dr R. R. Parker (Rocky Mountain Spotted Fever Laboratory, Hamilton, Montana, U.S.A.) has sent me a number of mites, and in this material amongst other interesting and possibly new specimens, there were a number of mites (all females) belonging to a new species of *Liponyssus* for which I propose the name *cynomys*.

In a recent letter Mr Hopkins drew my attention to a paper in *Ann. Mag. Nat. Hist.* by M. Perkins describing a new genus and species of Anoplura (*Acanthophthirius etheldredae*) which Dr Ferris had stated to be an immature mite. From Perkins's figure it was obvious that the specimen was a male *Myobia*. The allotype had been placed in the collection of the Molteno Institute, Cambridge, but subsequently given to the British Museum.

To all the above persons I owe my thanks for the ready assistance they have given me in access to the material used in the preparation of this paper. The allotypes and holotypes are placed in the National Collection, British Museum. Paratypes of *Laelaps buxtoni* have been placed in the collection of the London School of Hygiene and Tropical Medicine.

Genus **Laelaps** Koch, 1842

Laelaps buxtoni n.sp.

The most noticeable feature of this species is the shape of the anal scutum which is almost as broad as the genito-ventral scutum.

The female venter (Fig. 1) shows the usual arrangement of ventral scuta common to the genus; the sternal scutum is almost as long as broad, with the antero-lateral projections extending between coxae i and ii, a slight projection between coxae ii and iii, the postero-lateral corners rounded off, and the usual

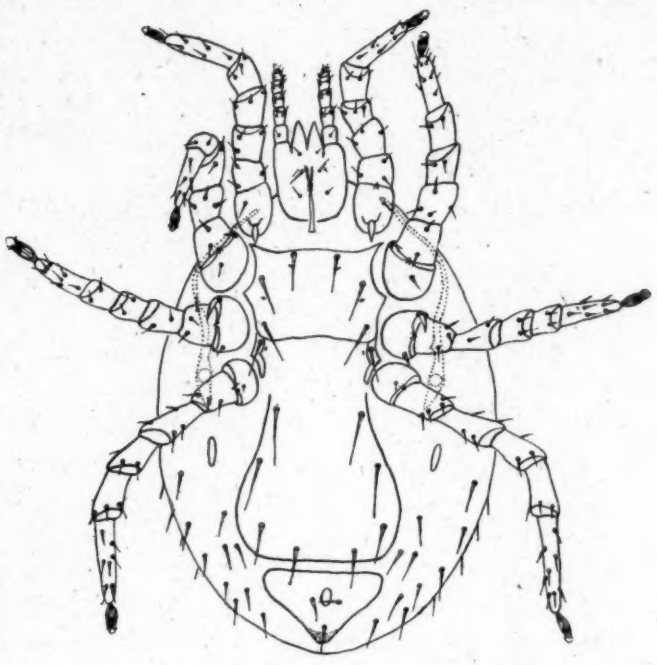

Fig. 1. *Laelaps buxtoni* n.sp. Venter of female.

three pairs of sternal spines and two pairs of lyriform pores. The metasternal scuta seem to be continuous with the sternal scutum, and each bears a spine on its anterior half.

The genito-ventral scutum is expanded behind the genital pair of spines, the posterior border being almost straight. The four pairs of spines are placed inside the border of the scutum with the fourth pair close together. Flanking the genito-ventral scutum and mid-way between it and the lateral edge of the body is a metapodal scutum, elliptical in shape. Close to the posterior border of the genito-ventral scutum is the anal scutum, which is widely triangular in shape,

with the anal pore situated in the centre, flanked by the usual pair of anal spines and with the unpaired anal spine lying well back towards the tip of the scutum.

In addition to the spines on the ventral scuta there are about ten pairs of spines situated behind the fourth pair of legs on the soft integument of the body. The peritreme extends from the level of coxa iv to the middle of coxa i.

The chaetotaxy of the legs has only two outstanding features, the stout thorn-like spine on the venter of coxae i, and the strong spur-like spine on the anterior edge of coxae iii.

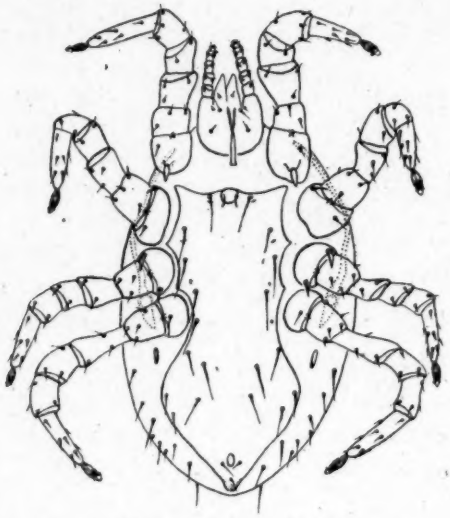

Fig. 2. *Laelaps buxtoni* n.sp. Venter of male.

The male venter (Fig. 2) shows the scutum to extend from the level of the anterior of coxa ii to the posterior end of the body, and bearing ten pairs of spines in addition to the three anal spines. In the specimen figured one of the genital pair of spines is missing. The anterior edge of the scutum has a concavity on each side of the genital pore, with antero-lateral projections between coxae i and ii. Slight projections of the scutum extend between coxae ii and iii, and between coxae iii and iv. The scutum is expanded behind the genital pair of spines, then tapers towards the anal pore. In addition there is on each side of this scutum an elliptical metapodal scutum, behind leg iv, and eight pairs of spines on the soft integument.

The chaetotaxy of the legs is identical with that of the female and the prominent thorn-like spine of coxae i, and the anterior spur-like spine of coxae iii are present.

The dorsum of the sexes is almost completely covered by the dorsal scutum, possessing a number of moderate spines and terminated at the posterior tip with a pair of long spines which are visible from the ventral aspect.

Host. A gerbille.

Locality. Madras, India.

Measurements. ♀0·45 × 0·32 mm.; ♂0·35 × 0·22 mm.

Genus **Haemolaelaps** Berlese, 1910

Haemolaelaps tachyoryctes n.sp.

The female venter (Fig. 3) shows the first pair of sternal spines to be placed on the anterior edge of the sternal scutum. There are lateral projections of the

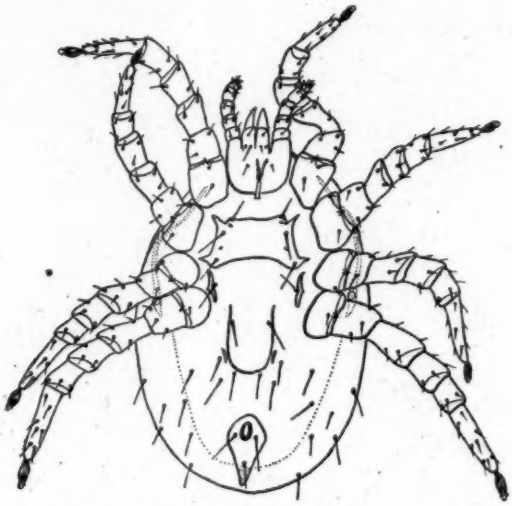

Fig. 3. *Haemolaelaps tachyoryctes* n.sp. Venter of female.

scutum between coxae i and ii and between coxae ii and iii, and the posterior edge of the scutum is concave. The second pair of sternal spines is placed well forward on the scutum, whilst the third pair is placed at the postero-lateral corners. The metasternal scuta are situated some little way from the sternal scutum and on the inner side of these scuta there is the metasternal spine. The genito-ventral scutum is placed well to the centre of the body and bears the genital pair of spines. Flanking this scutum is a pair of small scuta. The anal scutum is placed well back towards the posterior end of the body. It is pear-shaped with the anal pore close to the anterior border of the scutum, the paired anal spines being situated close to the posterior end of the pore. The soft integument of the body is furnished with ten pairs of spines as figured. The

peritreme extends from coxae iv to the middle of coxae i. Chaetotaxy of the legs is normal. The dorsal scutum is shown in dotted line on the figure and only covers the centre portion of the dorsum, leaving a wide border around it of soft integument.

The venter of the male (Fig. 4) shows the anterior edge of the ventral scutum to be concave on each side of the genital pore, with lateral projections between coxae i and ii, ii and iii, and between iii and iv. The scutum expands towards the lateral border of the body behind legs iv, and is then constricted just in front of

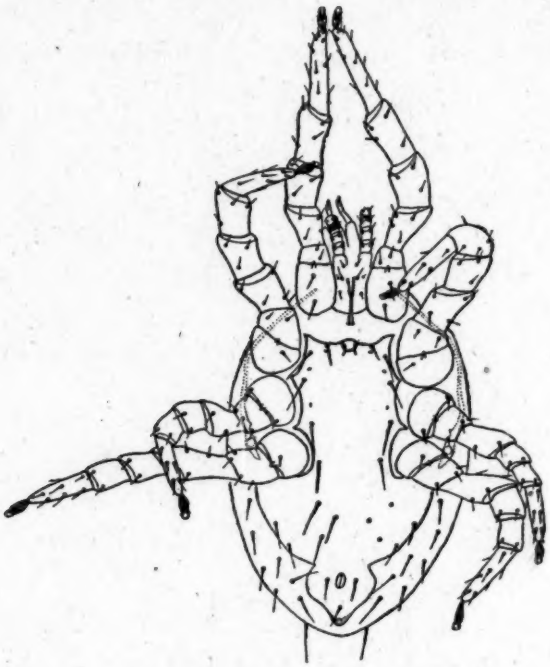

Fig. 4. *Haemolaelaps tachyoryctes* n.sp. Venter of male.

the anal pore. The scutum bears ten pairs of spines in addition to the three anal spines, and three pairs of lyriform pores. Three of the spines are missing in the specimen figured but the bases of the spines can be seen behind legs iv. The soft integument of the venter is furnished with seven pairs of spines between the last pair of legs and the posterior end of the body. Chaetotaxy of the legs is normal.

Host. Mole rat (*Tachyoryctes ruddi*).

Locality. Kapretwa, Mt Elgon, Kenya.

Measurements. ♀0·55 × 0·35 mm.; ♂0·4 × 0·25 mm.

Genus **Liponyssus** Kolenati, 1858

Liponyssus britannicus n.sp.

The venter of the female (Fig. 5) shows the sternal scutum to lie practically between coxae ii. The anterior edge is concave on each side of the central convexity, with antero-lateral projections between coxae i and ii. The lateral

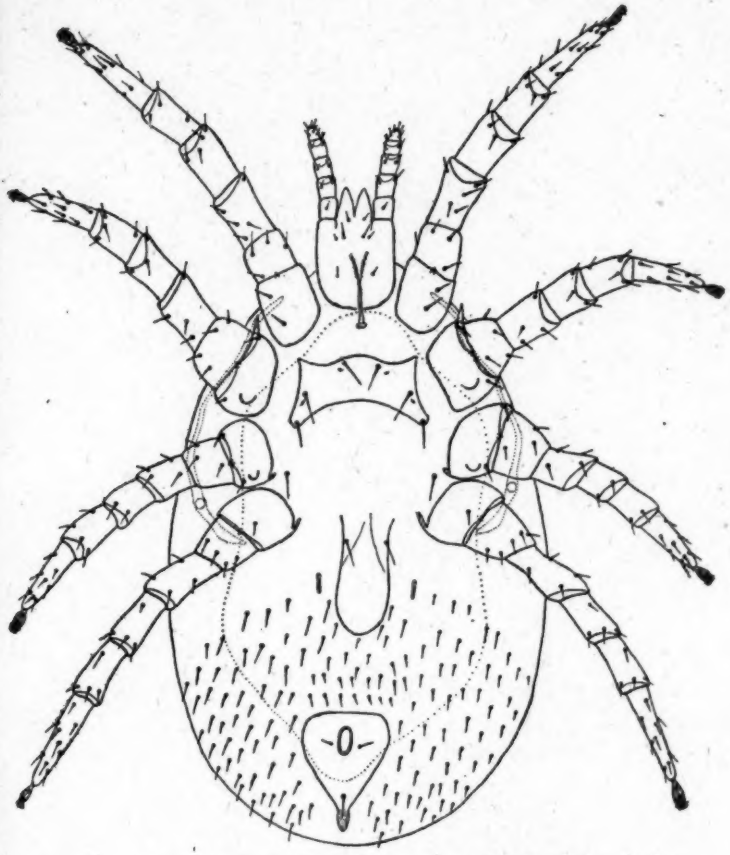

Fig. 5. *Liponyssus britannicus* n.sp. Venter of female.

and the posterior border are concave. The scutum is wider than long, with the first pair of sternal spines close to the centre of the anterior border, the second pair about midway down the lateral edge, and the third pair placed at the postero-lateral corners. The meta-sternal scuta are weakly chitinized or absent although the spines are present and are placed on a level with the

posterior edge of coxa iii. The genito-ventral scutum is long and narrow and bears the genital pair of spines. On either side of this scutum is a small elliptical scutum. The anal scutum is triangular with the antero-lateral corners rounded and the apex expanded to form a pointed projection. The anal pore is placed well forward on the scutum, the paired anal spines being placed behind the level of the middle of the pore. The soft integument of the ventral surface bears about seventy pairs of spines behind legs iv.

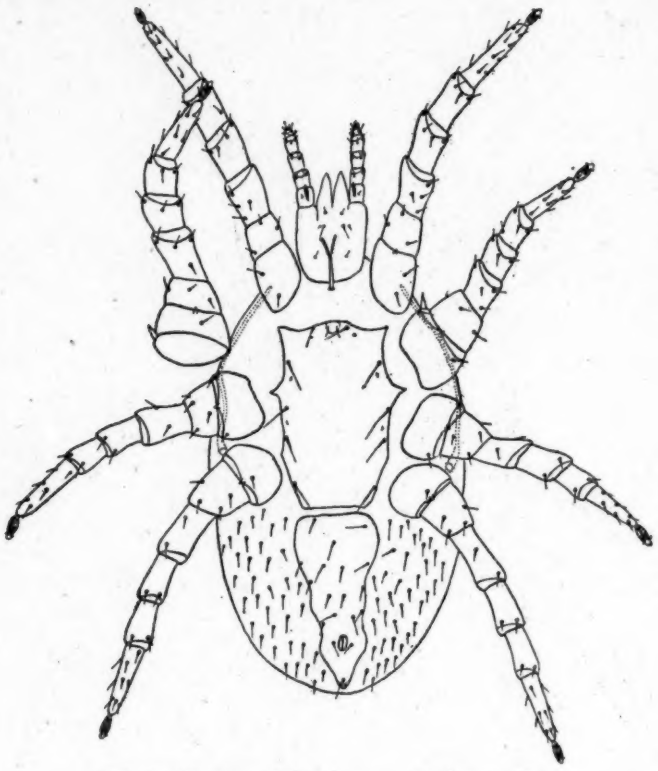

Fig. 6. *Liponyssus britannicus* n.sp. Venter of male.

The chaetotaxy of the legs is normal; on the anterior edge of the second coxa there is a prominent tooth-like spine, and on the ventral surface of coxae ii and iii there is a half-moon shaped chitinized process.

The dorsal scutum is shown in dotted line, which shows that a wide border of the dorsum is covered with soft integument.

The male venter (Fig. 6) shows the anterior border of the ventral scutum to be concave on each side of the genital pore, with the antero-lateral corners pro-

jecting between coxae i and ii. The lateral edge of the scutum has a projection between coxae ii and iii, and appears to have a suture behind the genital pair of spines. The posterior portion of the ventral scutum is furnished with fifteen spines in addition to the usual three anal spines. Behind legs iv and on the soft integument of the body there are thirty-seven pairs of spines. The tooth-like spine on the anterior edge of coxae ii is present in the male, and the chaetotaxy of the legs is normal.

Host. A bat (*Nyctalus noctula* Schreb.).

Locality. No data. Army School of Hygiene.

Measurements. ♀0·56 × 0·4 mm.; ♂0·45 × 0·27 mm.

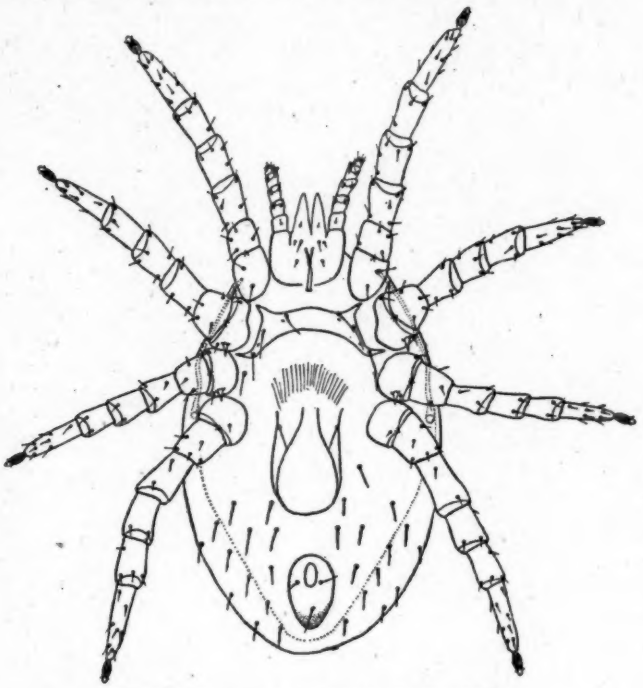

Fig. 7. *Liponyssus cynomys* n.sp. Venter of female.

Liponyssus cynomys n.sp.

The female venter (Fig. 7) shows the sternal scutum to lie between coxae ii, to be wider than long; the anterior border being convex centrally with a concavity on each side and the antero-lateral corner projecting between coxae i and ii. The lateral border of the scutum is almost straight to the postero-lateral corner which projects between coxae ii and iii. The posterior edge of the scutum is concave. Metasternal scutum absent, but the spines are present on a level with the middle of coxae iii.

Genito-ventral scutum, bearing the genital pair of spines, is widely separated from the anal scutum, which lies well to the posterior end of the body and is oval in shape. The paired anal spines are almost at the level of the posterior end of the anal pore which is situated at the anterior end of the scutum. The soft integument of the venter is furnished with fourteen pairs of spines.

Chaetotaxy of the legs normal with the exception of coxae ii and iii; there is a strong tooth-like spine anteriorly on coxae ii and a similar tooth-like spine posteriorly; on coxa iii there is also a tooth-like spine posteriorly.

The dorsal scutum (shown in dotted line) does not cover the entire dorsum, leaving a wide margin of soft integument from behind the last pair of legs.

Host. Prairie dog (*Cynomys* sp.).

Locality. Antonito, Colorado, U.S.A.

Measurements. ♀0·45 × 0·27 mm.

Genus **Myobia** von Heyden, 1826

Myobia etheldredae n.sp. (Perkins, 1925) emend. Radford.

This mite was originally described and figured by Michael Perkins as a new genus and species of Anoplura, but from his figure it was obviously a male *Myobia*. I have to thank the British Museum authorities for the loan of the allotype slide from which the present description and figure are compiled. The genital pore and penis are not visible.

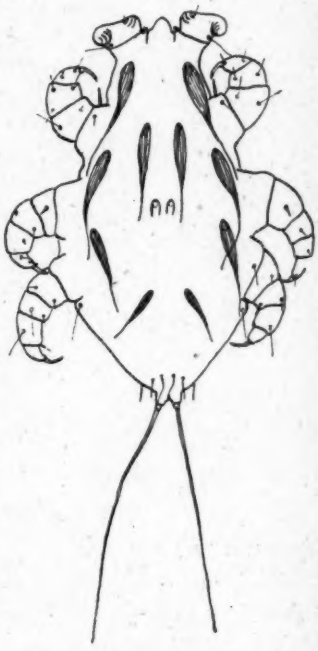

Fig. 8. *Myobia etheldredae*. Dorsum of male.

The male dorsum (Fig. 8) shows the usual arrangement of three outer pairs of spines, broadly expanded at their base and tapering to a slender point; striated longitudinally. The first pair of the outer series is placed some little distance in front of legs ii; the second pair is posterior to the level of legs ii, and the third pair is posterior to the level of the third pair of legs. The first pair of the inner spines is placed on a level with legs ii. A second pair of inner spines arises behind this first pair and level with legs iii, but the specimen has in this region a mass of undigested food which appears as an area of black granular matter through which it is not possible clearly to define the outline of the spines in the centre of the body. The next pair of these expanded, striated spines appears on a level with the last pair of legs. Close to the posterior end of

the body there is a line of six slender spines with the customary terminal bristles mounted on two nodules.

The chaetotaxy of the legs is normal for the genus with the exception of a stout spine on the dorsal surface of legs iii, on segment ii; on the ventral surface of this segment and the next two (3rd and 4th segments) there is a short dagger-like spine.

Host. A bat (*Pipistrellus pipistrellus* Schreb.).

Locality. Ely Cathedral.

Measurements. ♂ 0·5 × 0·2 mm.

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A NOTE ON THE BIONOMICS OF *IXODES RICINUS* L.

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During recent years several investigators have recorded their observations on the bionomics of the sheep tick, *Ixodes ricinus*, and our knowledge of the life cycle and habits of this parasite is becoming more detailed. Whilst maintaining stocks of living *I. ricinus* in the laboratory to make studies on their anatomy, it was considered that certain facts, emerging from the routine examination of the ticks, were of sufficient interest to place on record. An extensive study of the life history and habits of the tick was not attempted, and the object of this note is mainly to supplement the observations already recorded by other investigators.

After the living ticks were received on 17 April 1939, they were examined almost daily but, as the result of circumstances arising from the outbreak of war, they were later stored and were not observed for nearly 7 months. It was after the examinations had been resumed that the data recorded in this paper were revealed.

Technique

The ticks, in all their stages, were kept in well-stoppered 3 × 1 in. glass tubes, placed on damp cotton-wool in beakers. At first, the tubes were closed with cotton-wool plugs but were later firmly stoppered with corks, as moulds grew readily on the inner surface of the tubes and on the ticks themselves. About twice each week the corks were removed for a few minutes for ventilation. When the ticks were first received, as engorged females, they were placed in an incubator at 24° C., but later, as the weather became warmer, were kept in a cupboard at room temperature because it was found that moulds grew readily when the specimens were in the incubator. After oviposition, the larvae, which hatched from the eggs, were fed by being placed on the scrotum of a young ram.

At the outbreak of war, the larval and nymphal ticks were placed in tubes, which were packed in a wooden box, to await transport to other laboratory accommodation. As such they remained for nearly 7 months. When the specimens were eventually received, after this period, the tubes containing the ticks were replaced on damp cotton-wool in beakers and later feeding was recommenced. As a ram lamb was not available, guinea-pigs were used, pill boxes containing the ticks being attached to their shaved sides with adhesive

tape after the fashion described by Trager (1939). From experience, guinea-pigs were found to be unsatisfactory animals to use, and later, when hedgehogs became available, they were used instead.

OBSERVATIONS

Twenty-four female specimens of *Ixodes ricinus* originally received, eggs being obtained from eleven of them. Although larvae hatched from all the eleven batches of eggs, only five groups succeeded in reaching the stage of larval engorgement. The larvae in the remaining six groups were discarded because they either refused to feed or died soon after hatching. As to the five groups referred to above, the larvae in two of these, after engorgement, became dried and shrivelled during the winter months.

Table 1. *Temperature during hatching was* 18–20° C.

[illegible] of female	Oviposition began 1939	Oviposition ended 1939	Duration in days	Larvae emerged 1939	Time of hatching in days	Larvae placed on ram 1939	All larvae engorged by 1939	Nymphs emerged	Time from start of larval feed in days	Nymphs placed on guinea-pig 1940	All nymphs engorged by 1940
7	25. iv	23. v	29	4. vi	41	26. vi	3. vii	Larvae died during winter	—	—	—
8	1. v	3. vi	34	17. vi	48	27. vii	31. vii	Larvae died during winter	—	—	—
9	26. iv	31. v	36	6. vi	42	17. vii	22. vii	28. viii. 39 21. vi. 40	43 341	27. v 26. vii	3. vi 31. vii
13	29. iv	8. vi	41	2. vi	35	3. vii	10. vii	21. viii. 39 20. vi. 40	50 354	10. vi 26. vii	Batch lost 1. viii
14	25. iv	3. vi	40	31. v	37	19. vi	13. vii	20. viii. 39 13. vi. 40	63 361	3. vi 16. vii*	7. vi 21. vii

* This batch of nymphs was fed upon a hedgehog.

As previously mentioned the female ticks were received on 17 April 1939, and were maintained at an even temperature, in an incubator, of 24° C. until about the time when hatching of the eggs started. Reference to Table 1 shows that, until August 1939, the duration of the various developmental stages was that generally recorded under laboratory conditions. The dates given for larval and nymphal emergence are those on which the first larva or nymph in each batch appeared. Actually, hatching of all the larvae or nymphs in each batch occupied a number of days. It will thus be seen, from reference to the table, that some larvae resulting from eggs laid by females nos. 9, 13, and 14 had moulted into nymphs by August 1939, but by no means all of them.

The first group of nymphs resulting from each batch of larvae were fed upon guinea-pigs at the end of May and beginning of June 1940. During the second and third weeks of June 1940, however, the remaining larvae, which had engorged nearly a year previously, moulted into nymphs, which were normally active and, when fed upon hedgehogs, became engorged in 4–7 days. In the case of larvae resulting from female no. 14, as the batch contained a large number of specimens it was necessary to feed them in small groups over a

period of about 3 weeks and this explains the long interval, indicated in the table, between the date the ticks were placed on the ram and the time by which all had engorged.

Discussion

It is a well-known fact that *Ixodes ricinus* can survive for long periods in the fed as well as the unfed stages. MacLeod (1932) found that the longevity of unfed males and females was 21 months, one male remaining alive in the laboratory up to 31 months; unfed larvae were alive after 2 years. Thus, although the findings recorded in this paper are not altogether surprising, it is of interest to compare them with those of other investigators.

MacLeod (1932) found that, from larvae engorged in August and kept in the laboratory, nymphs emerged in 6–7 weeks, whereas larvae engorged in September and October passed the winter as larvae and emerged as nymphs after 28–37 weeks. The data recorded in this paper show that, from some larvae engorged in July, nymphs emerged after 6, 7 or 8 weeks, whereas the remaining larvae moulted to nymphs after 48, 50 and 51 weeks respectively. Mönnig (1938) states that 4–37 weeks, depending on the temperature, may elapse, and Nuttall (1911) calculates, on the basis of various observations, that 28–140 days are necessary before the larvae moult, after engorgement. Stockman (1911) records the overwintering of *Haemaphysalis punctata* as engorged larvae which did not moult until the following spring; the longest time observed between larval engorgement and ecdysis was 238 days.

In planning any methods of control based on the longevity of the ticks, the importance of the fact that, in times of adverse climatic conditions, the various stages may be very prolonged is here emphasized. Whereas the average period for larval metamorphosis is commonly calculated to be about 6 weeks, this time may be enormously extended even under natural environmental conditions.

The temperature and relative humidity of the atmosphere surrounding the ticks during their seven months' storage period was not determined. As the ticks were enclosed in corked glass tubes, placed within a wooden box, and there was no additional moisture available during this period, the specimens were probably subjected to a low humidity. Two batches of engorged ticks did, in fact, dry up during the winter months. From December 1939, until March 1940, the ticks were kept in their box in the laboratory, in which a coal fire burnt each day, but the unusually low air-temperatures experienced during the first quarter of 1940, combined with the low relative humidity, were probably the chief factors delaying ecdysis. The nymphs which eventually emerged in June 1940, fed on a hedgehog during the usual period of 4–7 days, and were recovered engorged. MacLeod (1932) records that larvae were able to feed after 15 months; and nymphs after 13 months of fasting.

SUMMARY

Observations have been made on the life cycle of *Ixodes ricinus*, whilst rearing stocks of this tick in the laboratory. Some larvae were found to undergo a prolonged metamorphosis into nymphs and periods of 341, 354 and 361 days between the start of larval engorgement and nymphal emergence are recorded. The rather exceptional conditions under which the ticks were kept are described as well as the methods of maintaining and feeding the specimens.

ACKNOWLEDGEMENTS. The writer is indebted to Mr W. Lyle Stewart, M.R.C.V.S., who kindly supplied the original specimens of *I. ricinus* and to Dr J. N. Oldham for his helpful advice and criticism.

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FURTHER STUDIES ON THE ULTRAFILTRATION OF PLANT VIRUSES

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(With 5 Figures in the Text)

Introductory

In a previous publication in this series (Smith & MacClement, 1940) we described the membrane filtration of *Nicotiana virus* 11, tobacco necrosis virus, and put forward certain suggestions to explain the peculiarity of the filtration curve. In the present paper we describe filtration studies with nine other plant viruses and show that there exists a small group of apparently spherical viruses which filter in a consistent manner. All the other plant viruses so far tested show a greater or less degree of divergence in their filterability from this small group.

Material and methods

The following viruses were used in the filtration experiments: *Nicotiana virus* 1 (tobacco mosaic virus), *Nicotiana virus* 1A (tobacco enation mosaic virus), *Nicotiana virus* 1C (aucuba mosaic virus), *Nicotiana virus* 1D (masked tobacco mosaic virus), *Nicotiana virus* 1E (an undescribed strain of tobacco mosaic virus resembling strain 1C), *Nicotiana virus* 12 (tobacco ringspot virus), *Lycopersicum virus* 4 (tomato bushy stunt virus), *Solanum virus* 1 (potato virus *X*) and *Cucumis virus* 1 (cucumber mosaic virus).

The viruses of the tobacco mosaic type were all cultivated in tobacco, var. White Burley, and the virus sap was subsequently extracted by mincing the leaves and pressing through fine cheesecloth. Clarification of the sap in the case of these viruses was achieved by passage of kieselguhr. *Nicotiana virus* 12, the tobacco ringspot virus, was cultivated in Turkish tobacco, var. Kawala, but the sap was clarified by spinning on the centrifuge since it was found that passage of kieselguhr greatly reduced the virus concentration of the sap. The virus suspension was adjusted to *p*H 7 and centrifuged, the supernatant fluid was then brought to *p*H 8·3 with *M*/10 phosphate buffer and soda and again centrifuged. This gave a clear suspension with high virus content.

Solanum virus 1 and *Cucumis virus* 1 were cultivated in tobacco, var. White Burley; sap containing the former virus was clarified by passage of kieselguhr and occasionally by centrifuging, while in the latter case the virus sap could only be clarified by centrifuging since this virus adsorbs onto kieselguhr.

Lycopersicum virus 4, causing the bushy stunt disease of tomato, is only known to become systemic in two host plants, the tomato with which it has been chiefly associated and the solanaceous weed, *Datura Stramonium*. The latter plant is rather unsuitable for obtaining large quantities of virus owing to

the mucilaginous character of the sap. It was necessary therefore to use the tomato as the source of virus and young rapidly growing plants were inoculated when about 6 in. high. As soon as the plants showed symptoms of systemic

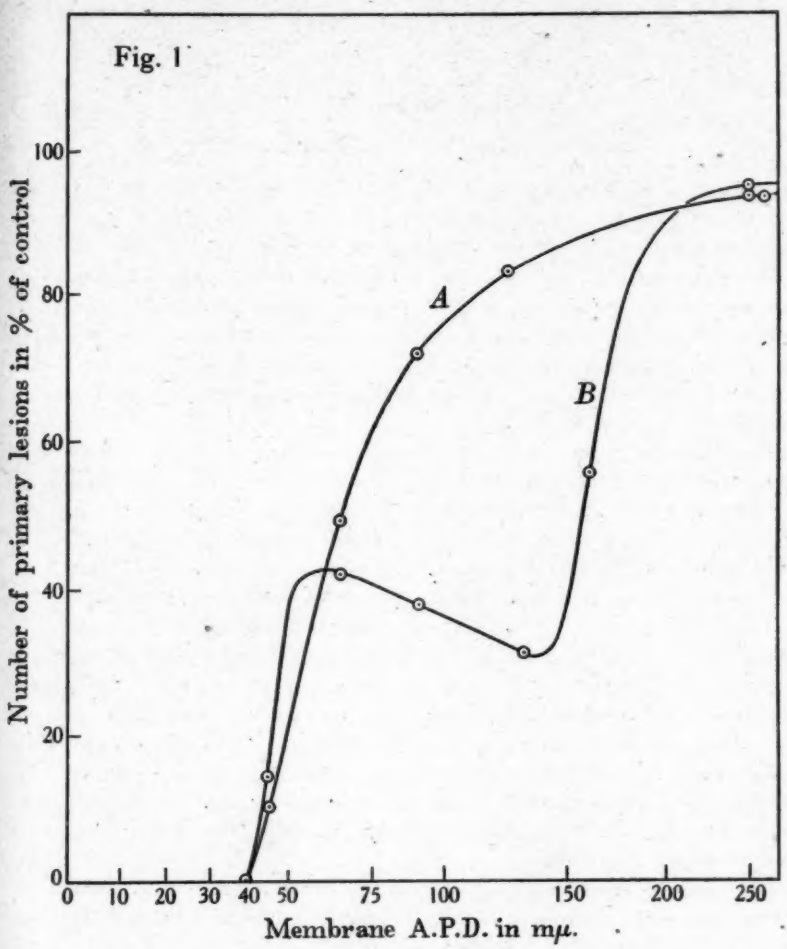

Fig. 1. *A*, the filtration curve for *Lycopersicum virus* 4 contrasted with *B*, the filtration curve for *Nicotiana virus* 11.

disease, they were gathered and the juice extracted as with the other viruses. The sap was clarified by passage of kieselguhr.

All viruses were filtered at a *p*H of 8·3 in a 4 : 1 virus-broth mixture, Hartley's broth being used. Before being mixed with the virus the broth was

filtered through a tight membrane of 20 mμ average pore diameter and 2 ml. of this broth were also passed through each membrane before filtration.

For the filtration studies of precipitated virus, various methods of precipitation were used. The tobacco mosaic group of viruses were all precipitated with 50% alcohol and *Solanum virus* 1 was precipitated at its isoelectric point (*p*H 4·5).

Experimental

Lycopersicum virus 4 (tomato bushy stunt virus)

This virus was first discovered and described by one of us a few years ago (Smith, 1935) and it does not appear to have been recorded outside the British Isles. In preliminary filtration experiments, described at that time, it was at once recognized that this virus was outstanding in its ease of filterability as compared with such a virus as that of tobacco mosaic or potato virus *X*. It was recognized also that it was the smallest plant virus to have been measured up to that time and the particle size was tentatively given as 17–25 mμ. Improvements in filtration technique have now shown that the actual figure is slightly smaller than this.

The filtration curve for *Lycopersicum virus* 4 is given in Fig. 2*B* and it is compared with 2*A*, the anticipated curve for a spherical particle filtering under optimum conditions. More details of the filtration experiments with this virus are given in Fig. 3, where each sign represents one filtration experiment. The plus sign indicates that the number of lesions produced on the test plant (*Nicotiana glutinosa*) exceeds 5% of the control while the multiplication sign indicates less than 5%; the circle shows that no virus passed the membrane. It will be seen that 97 filtration experiments were carried out with this virus and of these 68 were positive and the remaining 29 negative. The uniform filtration is indicated by the fact that the virus content of the filtrate does not fall off rapidly with descending pore size, and the circles and multiplication signs indicating loss of virus do not appear in the table until the region of the limiting A.P.D. is reached. Comparison of these filtration details with those obtained with most of the other viruses and shown in Figs. 3 and 4 will make the difference quite clear.

In order to investigate the filtration properties of precipitated virus some experiments were carried out on the filtration of a purified sample. This was kindly given us by Mr N. W. Pirie and it was used at a dilution of 1 : 20 to bring it to the same concentration as the unpurified virus. The filtration curve for the purified sample of *Lycopersicum virus* 4 is given in Fig. 2*C*. It will be seen that although the filtration end-point is the same as for the unprecipitated virus the slope of the curve is different, and this suggests that there may be a slight degree of aggregation.

Reference to Fig. 3 shows that filtration experiments with membranes of A.P.D. less than 40 mμ are all negative. This then is taken as the filtration end-

point of the virus, which by Elford's method of calculation gives a particle diameter for the virus of 13–20 mμ.

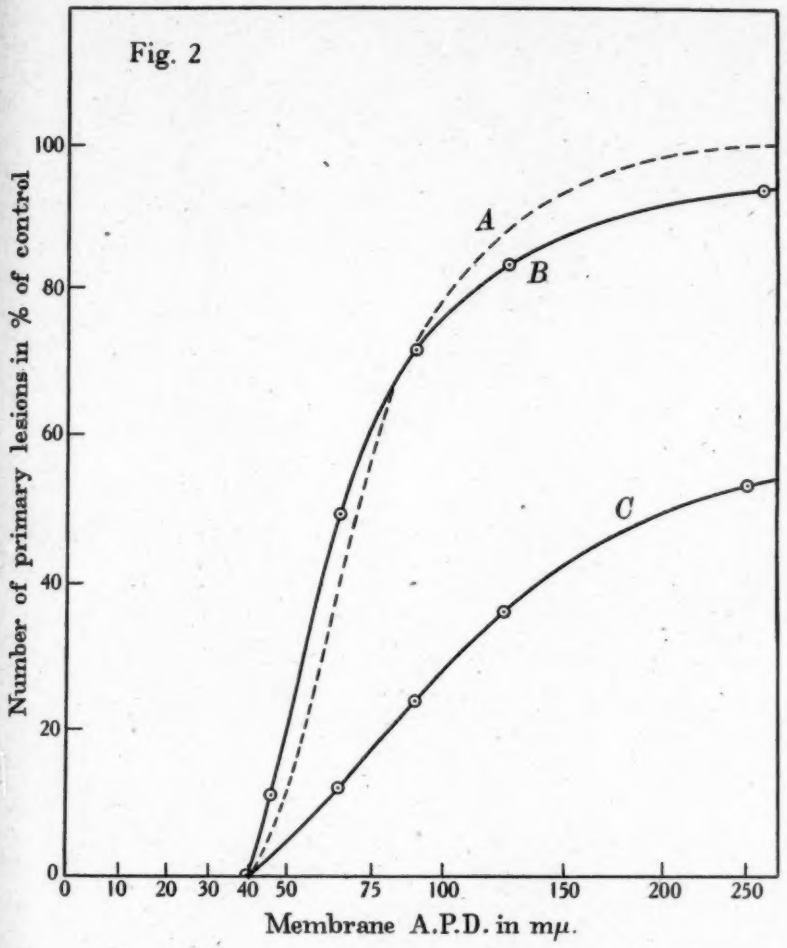

Fig. 2. Filtration of *Lycopersicum virus* 4. *A* is the anticipated curve for a monodisperse system filtering under optimum conditions. *B* is the curve for unprecipitated virus in clarified sap, and *C* is that for precipitated and purified virus.

Nicotiana virus 12 (tobacco ringspot virus)

A good deal of difficulty was experienced at first in filtering this virus in a satisfactory manner and the rather inconsistent results then obtained were considered to be due to the low initial virus content of the samples to be filtered. Furthermore, several of the methods of clarification of the sap which

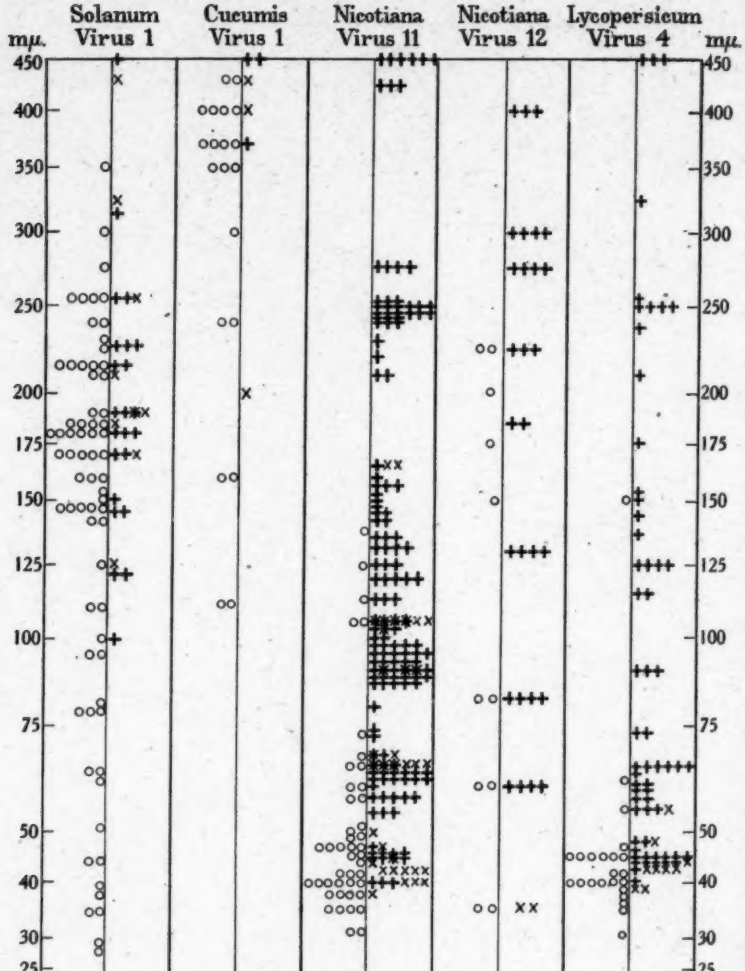

Fig. 3. Filtration data of five plant viruses. The plus sign indicates that the virus in the filtrate exceeded 5% of the control; the multiplication sign indicates less than 5% and the zero sign indicates that no virus passed the filter. Each sign represents one filtration experiment.

were used reduced still further the virus concentration of the samples. On the suggestion of Dr W. M. Stanley the method of clarification by changing the *p*H and then centrifuging as described on page 320 was tried. By this means a clear suspension of high virus content was obtained and under these conditions the virus filtered fairly consistently down to an A.P.D. of 40 mμ (see Fig. 3). This gives a particle size of 13–20 mμ, the same as for the tomato bushy stunt virus, and is in agreement with Stanley's results (Stanley, 1939).

Nicotiana virus 11 (tobacco necrosis virus)

The filtration peculiarities of this virus have been described in a previous publication (Smith & MacClement, 1940) and the filtration curve is given in Fig. 1 *B* for comparison with that of *Lycopersicum virus* 4 shown in curve *A*. Details of the filtration experiments, 233 in all, will be found in Fig. 3. As in the case of *Lycopersicum virus* 4, the negative experiments in which no virus passed the membrane all occur in the region of the filtration end-point, 40 mμ, which is the same as for the two preceding viruses.

These three viruses then, *Lycopersicum virus* 4 and *Nicotiana viruses* 11 and 12, form a group in which the particle is apparently approximately spherical and in which the size is the same, 13–20 mμ. It is possible, however, that there exists some degree of dissymmetry of particle shape in the case of *Nicotiana virus* 11 (Smith & MacClement, 1940).

Nicotiana virus 1 and its strains

When we come to consider the filtration experiments with this group of viruses, we find a different state of affairs. In Fig. 4 are given the results of the filtration of *Nicotiana virus* 1 and strains 1A, 1C, 1D, and 1E. In the case of *Nicotiana virus* 1, 154 filtration experiments were performed and of these no fewer than 98 were entirely negative. It will also be noticed that the plus signs which indicate a fairly high virus concentration in the filtrate do not occur below the 100 mμ level.

Although filtrations between 150 and 200 mμ were frequently negative, further experiments showed that some virus continued to pass the membranes down to an A.P.D. of 40 mμ, which seems to be the filtration limit, at all events by this method. Twenty-one experiments with membranes of A.P.D. less than 40 mμ gave negative results (see Fig. 4).

The filtration experiments with the four other strains of *Nicotiana virus* 1 gave rather similar results. In the case of *Nicotiana virus* 1E, out of twenty-seven filtrations through membranes of A.P.D. less than 100 mμ only seven in the region of 60–80 mμ were positive. Of the other three strains, *Nicotiana virus* 1D seemed to filter slightly less readily, although fewer experiments were performed with these three viruses. It is clear that below an A.P.D. of 100 mμ filtration of the tobacco mosaic group of viruses is difficult and irregular.

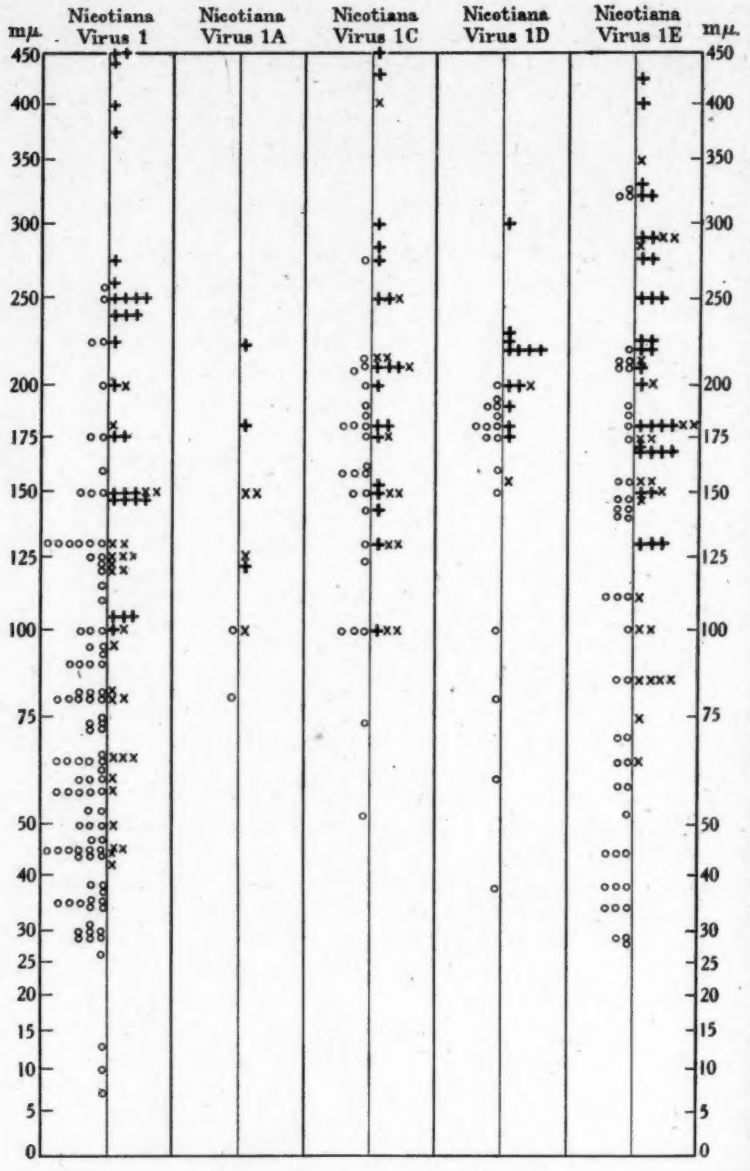

Fig. 4. Filtration data of *Nicotiana virus* 1 and its strains. Symbols as in Fig. 3.

Solanum virus 1 (potato virus *X*)

It will be seen from Fig. 3 that the filtration of *Solanum virus* 1 is as irregular as that of the tobacco mosaic group of viruses and that it falls into the same

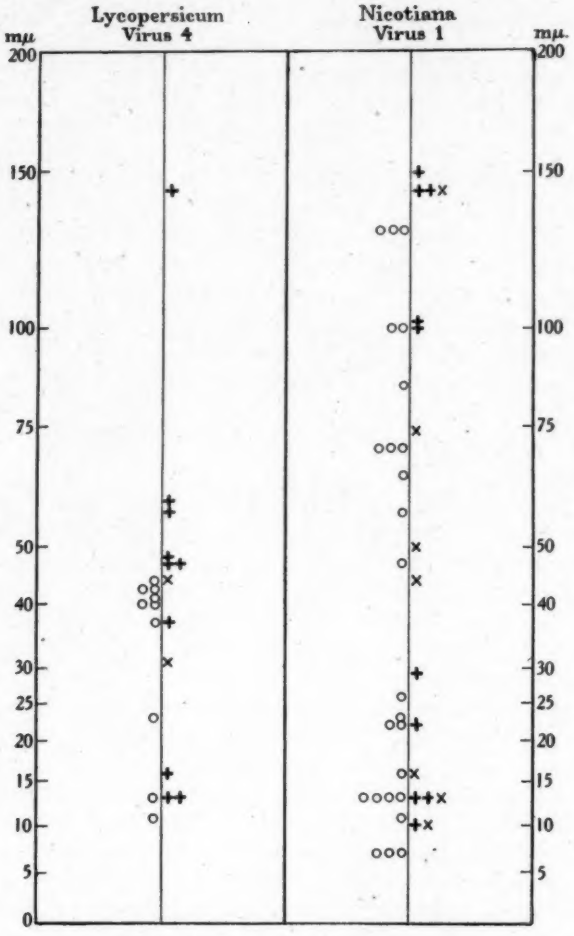

Fig. 5. Details of cataphoresis experiments. Symbols as in Fig. 3.

category. Out of 101 filtrations performed, sixty-nine were negative, and many of these failures occur well above the presumed filtration end-point of the virus, which we have found to be in the neighbourhood of 100 mμ.

Cucumis virus 1 (cucumber mosaic virus)

Like other viruses of this type, such as *Solanum virus* 2 (potato virus *Y*), cucumber mosaic virus is very difficult to filter and no conclusions as to filtration end-point or particle size have been reached. Out of twenty-six filtration experiments only six were positive and all these were in a region obviously above the true filtration end-point (Fig. 3). Some at least of the difficulty experienced in filtering this virus is due to lack of concentration of the virus in the sap and to its high capacity for adsorption.

Cataphoresis

Using a simple cataphoresis apparatus experiments were made whereby a virus could be drawn by means of an electric current through a membrane interposed between anode and cathode. Three viruses were studied in this way, *Nicotiana virus* 1, *Solanum virus* 1 and *Lycopersicum virus* 4. In the case of the first named virus, positive infections were obtained after passage of membranes of A.P.D. 10–15 mμ, compared to 50 mμ using positive pressure. Similarly *Lycopersicum virus* 4 seemed to pass a membrane of A.P.D. 13 mμ compared to 40 mμ with positive pressure (see Fig. 5). Negative results were consistently obtained in the case of *Solanum virus* 1, no movement through the membranes being observed even when discs of quite large A.P.D. were used.

Discussion

The results of the large number of filtration experiments described in this paper show very clearly the difficulties encountered in arriving at an accurate estimate of the particle sizes of plant viruses by this method. It can be stated with some confidence that three out of the total number of viruses studied are spherical in shape or nearly so, and the estimation of their particle size is in all probability fairly accurate.

As regards the group of tobacco mosaic viruses and *Solanum virus* 1 (potato virus *X*), however, the situation is entirely different. We are dealing here with viruses which are asymmetrical and probably rod-shaped. Moreover, the infective units appear not to be of constant size and the filtration results suggest that the variability is very great.

So far as tobacco mosaic virus is concerned the filtration end-point under positive pressure seems to be about 40 mμ; this gives an estimated particle size of 13–20 mμ. However, since the virus is considered to be rod-shaped, it seems likely that this value applies only to the diameter of the particle.

We do not know much about the relationship of particle size to pore size under the conditions of the cataphoresis experiments, but it may be presumed that the electric current would draw the virus through a smaller pore than would be possible by pressure, so that these latter results are also not inconsistent with an estimated particle diameter of 13–20 mμ. This value fits in

very well with the data obtained by other methods. Bernal & Fankuchen (1937), using X-rays, give a value for the cross-section of 15·2 mμ, Langmuir & Schaefer (1937) find it to be 12·5 mμ by their studies on monolayers of the virus. Lauffer (1938) obtains a value of 12·3 mμ by estimating molecular weight from viscosity, sedimentation and diffusion data, while Kausche *et al.* (1939), using the electron microscope, find the diameter and length of the particle to be 15 and 300 mμ respectively.

With regard to *Solanum virus* 1 (potato virus *X*) there is less agreement between the results achieved on particle size by filtration and other methods. It is possible to obtain some idea of the relative dimensions of rod-shaped particles from viscosity measurements and then from the relative viscosity to calculate the dissymmetry and molecular weight. From the weights and dissymmetry constant the lengths and widths can then be calculated. Using these methods Loring (1938) calculates that the virus would have a diameter of about 9·8 mμ and a length of about 433 mμ. From the filtration results described in this paper, however, a value of 33–50 mμ for the particle diameter is obtained if the filtration end-point is accepted as 100 mμ.

No deductions of any value can be made concerning the particle size of *Cucumis virus* 1 (cucumber mosaic virus) until it can be filtered in much greater concentration than that in which it occurs in the infected plant.

SUMMARY

An account is given of ultrafiltration studies with 9 plant viruses. It is shown that 3 of these viruses filter in a consistent manner and appear to have approximately spherical particles. These three are *Lycopersicum virus* 4 (tomato bushy stunt virus), *Nicotiana virus* 11 (tobacco necrosis virus) and *Nicotiana virus* 12 (tobacco ringspot virus). The filtration end-point of 40 mμ is the same in each case and from this a particle diameter of 13–20 mμ is calculated. There is a peculiarity, however, in the filtration curve of tobacco necrosis virus which shows itself in a "bench" or "shelf" and which suggests either a polydisperse system or some degree of dissymmetry of particle shape.

Great difficulty was experienced in filtering *Nicotiana virus* 1 (tobacco mosaic virus) and its strains. A value of 13–20 mμ was obtained for the particle diameter of the type virus and this agrees well with measurements obtained by other methods. The filtration results, however, suggest that the infective units are not of the same length and that this variability may be considerable. Similar difficulty was experienced in filtering *Solanum virus* 1 (potato virus *X*), another rod-shaped virus; the end-point was found to be 100 mμ, from which a particle diameter of 33–50 mμ is calculated. It was not possible to obtain a definite filtration end-point for *Cucumis virus* 1 (cucumber mosaic virus), probably because of the low initial concentration of virus in the extracted sap.

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THE CATTLE LICE OF GREAT BRITAIN

PART I. BIOLOGY, WITH SPECIAL REFERENCE TO *HAEMATOPINUS EURYSTERNUS*

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The Cooper Technical Bureau, Berkhamsted

(With 1 Figure in the Text)

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1. Introduction

Cattle lice have been known since the early ages, but only four papers (Lamson, 1917, 1918; Shull, 1932; and Blagovestchensky & Serdukove, 1935) give any details of the life histories of these parasites. Numerous papers deal with the control measures, some of which contain incidental observations on the biology of the lice.

The work carried out at this Research Station was designed to fill some of the gaps in our knowledge with the hope that such studies might lead to practical advances in control measures. It has been done in three phases: first, critical observations of the behaviour of the insects on the host and in the laboratory; secondly, observations on the natural incidence of lice populations on cattle; and thirdly, a survey based on a questionnaire sent to farmers in England, Scotland and Wales. Throughout this paper, and any subsequent papers upon the same subject, the term 'questionnaire' or 'the farmer's opinion' refers to the information obtained from this survey.

In Great Britain 84·5 % of the 309 farmers who replied to the questionnaire stated that their cattle were subject to lice infestations; 74·5 % of the farmers who admitted having lice on their cattle, stated that they can be considered a pest.

2. Geographical distribution in Great Britain (excluding Ireland)

There are three species of sucking lice in Great Britain. *Haematopinus eurysternus* Nitzsch, *Linognathus vituli* Linnaeus, and *Solenopotes capillatus* Enderlein, and one species of biting louse, *Bovicola bovis* Linnaeus. *Haematopinus tuberculatus* has been recorded as a parasite of cattle but not in this country.

H. eurysternus, *L. vituli* and *B. bovis* were originally recorded in Great Britain by Stephens (1829), but *S. capillatus* was not recognized in England until 1923 by Pillers and in Scotland by Craufurd-Benson (1938). 105 samples of lice were sent to the Research Station by some of the farmers who answered the questionnaire, the records (see Map, Fig. 1) giving an indication of the relative frequency of each species as observed by them.

Bovicola bovis	52
Haematopinus eurysternus	33
Linognathus vituli	16
Solenopotes capillatus	4

It appears from these figures and from personal observations that *B. bovis* is the commonest species in Great Britain, and is the most widely distributed. The evidence given in a later paper will show that although *B. bovis* is the commonest, and is observed in very large numbers, its economic importance is actually less than that of *H. eurysternus*. The distribution of the records of *H. eurysternus* indicates a decrease in importance of this species from south to north of Great Britain; this suggestion is discussed in Part II of this series.

The sixteen records of *L. vituli* are grouped in areas of high rainfall and where dairy cattle are most prevalent. Roberts (1938) stated that this species is more commonly found on dairy cattle than beef cattle.

S. capillatus is seldom observed by farmers, probably because of its small size, but is much more widely distributed than is realized at present (Roberts, 1938; Craufurd-Benson, 1938).

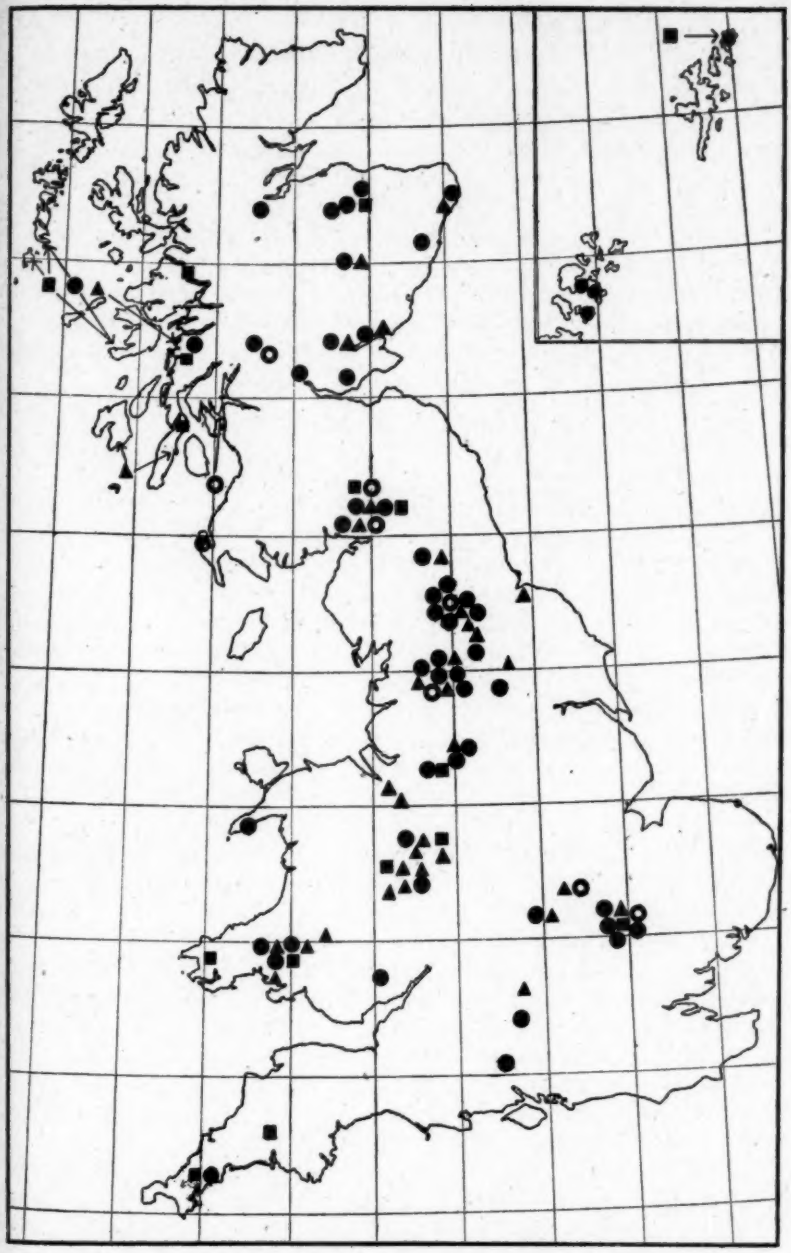
The questionnaire showed that the distribution of lice in Great Britain is not uniform. It appears that cattle in east England and east Scotland are extremely lightly affected, while cattle in southern Argyll, the Pennine Chain area, Wales, Monmouthshire, and the western and midland plains of England are more severely affected. A more detailed discussion of this evidence is given in a later paper.

3. Life histories

(*a*) *Methods*

All observations on the life histories of the various cattle lice have been made on young cattle, from 9 to 18 months old.

Small aluminium cells, similar to those described by Downing (1936), were used for confining the lice in small patches of hair. The hair around the central



● *B. bovis*; ▲ *H. eurysternus*; ■ *L. vituli*; ○ *S. capillatus*

Fig. 1. Records of cattle lice in Great Britain.

patch was clipped short so that the stubble could be forced into the cement while it was warm. This assisted in holding the cell in position. All cells were fixed on the flat areas of the rump and back.

In cold weather a cement of 80 parts rosin and 20 parts beeswax was used, and in the spring or autumn, or warm spells in winter, a cement of 80 parts rosin, 15 parts beeswax and 5 parts wool-grease.

For observations on the eggs, a number of female lice, with or without males, were placed in a cell and removed 24 hr. later. For recording the length of the various instars, other than the first instar, young nymphs were placed in the cell and the time of each instar recorded from moult to moult. The experiments on the rate of oviposition were made with female lice bred from the third instar.

(*b*) *The egg*

H. eurysternus eggs are somewhat pointed at the base, and measure 1·09 mm. (av.) long and 0·51 mm. (av.) at the widest part just below the rim of the operculum. They are hard shelled, unlike any of the other cattle-lice eggs, and usually an opaque white in colour, although brownish white or brown eggs are sometimes laid. The colour is no indication of the age or fertility of the egg. Eggs of *L. vituli* and *S. capillatus* are similar to each other, being dark blue with soft transparent egg shells. They are of an elongated oval shape, measuring, in the case of *L. vituli*, 0·95 mm. (av.) long and 0·39 mm. (av.) wide, and in the case of *S. capillatus* 0·76 mm. (av.) long and 0·32 mm. (av.) wide. The eggs of *B. bovis* are easily distinguished from those of the other cattle lice, as they are small, 0·65 mm. (av.) long and 0·29 mm. (av.) wide, and with a thin transparent egg shell. Fertile eggs have a glistening white appearance, and just prior to hatching, parts of the young embryo can be seen through the egg shell.

The eggs of all four species are laid near the skin, the distance from the skin varying in each case. Sometimes two, three, or even more eggs are laid on one hair, and the eggs are usually in clusters. *H. eurysternus* and *B. bovis* have definite egg-laying areas where the females congregate and lay their eggs in clusters. The distribution of these areas is discussed in Part II.

Table 1 shows the incubation period of *H. eurysternus* eggs on heifers at different times of the year. The incubation period of *B. bovis* eggs was observed at 7–10 days, av. 8 days (23 observations), *L. vituli* as 10–13 days (4 observations) and eggs of *S. capillatus* as 10–13 days (4 observations).

While these results agree with the records of Shull (1932), Bishopp (1921) and Imes (1925), they are appreciably longer than the periods observed by Lamson (1918).

Table 1 shows a correlation between the length of the incubation period of *H. eurysternus* eggs and the minimum air temperature, the higher the temperature the shorter being the incubation period. A similar correlation is believed to occur with *B. bovis*, but further results are required.

The findings of other workers on other species of lice have generally indicated a relation between temperature and the developmental period of the

Table 1. *Incubation period of* Haematopinus eurysternus *eggs*

Date eggs laid	No. laid	No. hatched	Incubation period (no. hatching per day)											Av. inc. period in days	Air temp. ° F.		Air humidity (R.H.)		Remarks
			9	10	11	12	13	14	15	16	17	18	19		Av. min.	Av. max.	Min.	Max.	
2. xi. 37	4	3	—	1	1	—	—	1	—	—	—	—	—	11	43·3	52·5	—	—	
27. xi. 37	12	9	—	—	—	—	1	6	1	—	—	1	—	14	35·4	44·2	—	—	
18. xii. 37	9	7	—	—	—	—	4	3	—	—	—	—	—	13	38·4	44·5	—	—	
24. xii. 37	16	13	—	—	2	7	2	—	2	—	—	—	—	12	39	44·1	—	—	
31. xii. 37	24	21	—	—	7	9	3	—	2	—	—	—	—	12	38·5	44	—	—	
2. ii. 38	17	9	—	—	—	—	—	—	2	5	1	—	1	16	37·6	45·3	60	84	Last 9 days av. min. temp. 31·9° F.
27. ix. 38	7	5	—	—	4	—	—	1	—	—	—	—	—	11	43·8	50·5	—	—	Naturally laid on calf's head
1–10. xi. 38	43	37	3	13	12	8	1	—	—	—	—	—	—	10–11	48·5	56	70	88	
9. ii. 39	15	13	—	—	2	9	2	—	—	—	—	—	—	12	42·6	50·3	80	93	Hair in cell thin to see if hatching prolonged
28. v. 39	32	14	—	3	8	1	2	1	—	—	—	—	—	11	52·5	75·2	49	76	
Total	180	131	3	17	36	34	15	12	7	5	1	1	1	12	—	—	—	—	

eggs (vide Florence, 1921; Nuttall, 1917; Bacot & Linzell, 1919; Blagovestchensky & Serdukove, 1935).

(c) *Post-embryonic life history*

A summary of the various observations on the length of the instars and pre-oviposition period of *H. eurysternus* is given in Table 2. The records were obtained during the winter months and in June 1938. No seasonal variation in the length of the various periods was observed, and no correlation was obtained with the air temperatures. On seven occasions the complete cycle from egg to adult was recorded, in which, except for the usual wide variation in the incubation period, the duration of each instar closely approximated to the average periods recorded in Table 2. No records were obtained for *B. bovis*, as all stages of this louse escaped from the cells. *L. vituli* and *S. capillatus* nymphs failed to survive confinement.

Table 2. *Duration of each instar and pre-oviposition of* Haematopinus eurysternus

	Length in days						Av. length in days
Period of life cycle	2	3	4	5	6	7	
1st instar	—	5	16	16	—	—	4
2nd instar	—	8	16	3	—	—	4
3rd instar	1	5	8	7	3	2	4
Pre-oviposition period	3	7	11	1	—	1	3–4

Table 3. *Complete life cycle of* Haematopinus eurysternus, *in days*

	Shortest possible time	Longest possible time	Computed average time	Lamson (1917)	Imes (1925)	Blagovestchensky & Serdukove (1935) *H. tuberculatus*
Incubation period	9	19	12	7–8	11–18 av. 14	Not less than 10
1st instar	3	5	4	—	—	3–5
2nd instar	3	4	4	—	—	3–4
3rd instar	3	6	4	—	—	3–4
Pre-oviposition period	2	7	4	—	—	3
Egg—adult	18	34	24	—	—	—
Larva—adult	9	15	12	14–17	12	9–11
Complete cycle—egg to egg	20	41	28	22–24	23–30	Not less than 21

(d) *Complete life cycle of* H. eurysternus

The complete life cycle of *H. eurysternus* can be computed from the various records, and from the seven complete cycles observed. This has been done in Table 3 and the results of other workers are included for comparison.

The observations of Blagovestchensky & Serdukove (1935) on *H. tuberculatus* are of particular interest as, although this louse has not been found in Great Britain, it is morphologically almost identical with *H. eurysternus*.

4. The biology of *Haematopinus eurysternus*

(a) *Sex ratio*

In the course of this work, sixty-one adults have been bred from the third instar larva, of which twenty-six were males and thirty-five were females, indicating an initial male : female sex ratio of 1 : 1·3.

The potential sex ratio, i.e. the relative proportions of the sexes in the third instar nymphal stage, has not been ascertained, as there was no reliable method of identifying the sexes in the final instar. The actual sex ratio, i.e. the ratio of males and females in a normal population of lice, have been obtained by counting all the lice on an animal. Eight of these counts have been made at different times, but not on different populations of lice on the same animal. The actual sex ratios obtained varied from 1 : 3 to 1 : 6·6, average 1 : 4.

It is obvious that at any time of the year the initial sex ratio is not the same as the actual sex ratio, i.e. 1 : 1·3 and 1 : 4 respectively. Although the evidence is not sufficient to allow any further conclusions to be drawn, there is a suggestion that the sex ratio, initial or actual, does not vary at different times of the year.

One count of the number of lice in a typical colony of *B. bovis* showed a male : female actual sex ratio of 1 : 24. The potential sex ratio was not recorded but the potential females easily outnumbered the potential males. Bedford (1932) remarked upon the relative scarcity of males of *B. bovis* under South African conditions. The general conclusions from the counts of the various populations are discussed in a later paper.

The disparity between the initial sex ratio and the actual sex ratio in colonies of *H. eurysternus* suggests that the female enjoys a longer span of life than the male.

(*b*) *Longevity*

(i) *On the host.* The longevity of the male of *H. eurysternus* on the host is very variable, the maximum recorded being 10 days; it appears to depend upon whether the male has succeeded in fertilizing a female. The male is capable of fertilizing more than one female.

The greatest recorded longevity of the female was 16 days. The sex ratios discussed earlier, indicated that there might be a differential longevity of males and females. A theoretical estimate of the average longevity of each sex can be made from the results of the counts made of lice populations. The most reliable count, by virtue of the numbers of lice counted, was made in November 1938. If it is assumed that the initial sex ratio was 1 : 1·3, that the population of lice at the time of the November count was static, that is to say, it was not rapidly increasing or decreasing, and that the average rate of development from the hatching of the egg to the emergence of the adult is 12 days, the calculation can be made as shown below:

Total number of	♂♂	...	80
" "	♀♀ ...	...	289
" "	nymphs	...	635
" "	lice	...	1004

Life of nymphs from hatching to emergence of adults 12 days.

If the population is static, the number of adults emerging per day is a twelfth of the existing nymph population, i.e. $\frac{635}{12} = 52{\cdot}8$. The initial sex ratio is 1 : 1·3 or 3 : 4, therefore number of males emerging per day is $\frac{52{\cdot}8 \times 3}{7} = 22{\cdot}6$. Number of females emerging per day is $\frac{52{\cdot}8 \times 4}{7} = 30{\cdot}2$.

Daily emergence of males ... ... 22·6
Total number of males 80
Average longevity of males, in days ... $\frac{80}{22·6}=3·5$

Daily emergence of females ... ... 30·2
Total number of females 289
Average longevity of females, in days ... $\frac{289}{30·2}=9$

Blagovestchensky & Serdukove (1935) give 16 days as the maximum longevity of a male of *H. tuberculatus*, and 22 days for the female, when both were kept and fed on man.

(ii) *Off the host.* The tests of longevity of lice off the host were complicated because, first, it is sometimes extremely difficult to determine when a louse is dead and, secondly, when replaced on the host, surviving lice move about, but die within a few hours, often without feeding.

Table 4. *Longevity of* Haematopinus eurysternus *off the host*

	Stage of lice	24 hr. starvation		48 hr. starvation		72 hr. starvation		96 hr. starvation	
		No. tested	% alive	No. tested	% alive	No. tested	% alive	No. tested	% alive
Lice kept at at 20° C. 70 R.H.	Males	20	75	36	33	20	0	—	—
	Females	100	99	100	28	100	0	50	0
	Nymphs	100	88	100	27	100	11	50	0
	Remarks	♀♀ laid 93 eggs, of which 90 hatched. Many nymphs moulted		♀♀ laid 35 eggs, of which 2 hatched. Some nymphs moulted		Few nymphs moulted		—	
Lice kept at 0–10° C. 70–85 R.H.	Males	30	54	28	28·6	20	0	—	—
	Females	50	72	74	9	50	0	—	—
	Nymphs	30	63	131	31	50	0	—	—
	Remarks	♀♀ laid 47 eggs, of which 42 hatched. Some nymphs moulted		♀♀ laid 0 eggs. Few nymphs moulted		—		—	

The method used for estimating longevity was to put the lice in cells on an animal's back after a given starvation period, and re-examine the lice 24 hr. later. Any lice that were obviously alive at the time of being put into the cell, but failed to live for 24 hr. on the host, were counted as 'dead' at the time of replacement. Thus, the results (see Table 4) do not show the actual longevity of the lice, but the period of starvation which renders the louse incapable of resuming normal life.

The tests were made with lice of unknown age that had fed previous to the starvation period. The lice were kept in glass tubes, either in an insectary at 20° C. and 70 R.H., or in the cowstall where the temperature and humidity fluctuated between 0 and 10° C. and 70–85 R.H. respectively. No attempt was made to distinguish the three nymphal instars of *H. eurysternus*, as the identification could be made only by relative size. The tests did show, however, that

the young nymphs in the first instar, and probably some of the second instar, were not so resistant to starvation as the older nymphs. The percentages of adults/nymphs of *B. bovis* surviving starvation at 0–10° C. 70–85 R.H. were:

24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.
94/82	91/80	68/30	26/3	5/0	0/0

Adults and nymphs of *S. capillatus* failed to survive 24 hr. starvation, but the result is not valid, as it is believed that this louse will not live a normal life when confined in cells on the back or rump of a heifer.

Imes (1925) states that *B. bovis* will withstand 7 days' starvation, the sucking lice about 4 days, and 'newly hatched lice live only 2 or 3 days unless they find a host'. The last remark implies that the eggs of lice will hatch off the host and presumably at a fairly low temperature. The present work has shown that no eggs of any of the cattle lice will hatch at any temperature below that usually obtaining on the host, and that newly hatched nymphs of *H. eurysternus* will not survive 24 hr. starvation at any temperature above 27·5° C. Bishopp & Wood (1917) state that *B. bovis* will live 7–15 days off the host.

(*c*) *Fertility*

The female can be fertilized by the male within a very short period of emergence. Several pairs of lice in copula were observed, and in a few cases the females were still very pale in colour, denoting that they could not be more than a few hours old.

Table 1 shows that of 180 eggs laid in metal cells, 131 hatched, a fertility of 72·8 %. The results were obtained during the period September to April 1937–8 and 1938–9 and in June 1938.

A large number of eggs were used for critical work in the laboratory. When they were kept at a suitable temperature for normal development, 80–90 % hatched. When the temperature conditions were unfavourable, the percentage of eggs hatching fell sharply. The fertility of the eggs, as distinct from the survival percentage, did not appear to vary at any time throughout the year.

(*d*) *Oviposition*

The female of *H. eurysternus*, whether fertilized or not, usually starts to lay eggs 4 days after emergence. The shortest pre-oviposition period was 2 days, the longest 7 days, and the average 4 days (Table 2). The pre-oviposition period did not appear to fluctuate at different times of the year or with the daily air temperatures, although a high daily temperature sometimes reduced the period.

Under natural conditions the egg-laying females usually cluster together and lay their eggs in masses. In the metal cells, the density of the females did not affect the rate of oviposition even when twenty were put together.

For the rate of oviposition tests, a newly emerged female was isolated with a male. As soon as oviposition started, the male was removed. In many cases the male was found dead as soon as the female started laying eggs. The maximum length of oviposition recorded was 15 days. It is possible that the

females might have been able to continue egg laying for a greater period if they had been allowed to be refertilized. In two cases an egg-laying female was observed to be in copula.

The maximum number of eggs laid by one female was twenty-four, but it is likely that this number can be exceeded. Three females, which escaped from their cells in November 1938, had laid twenty-one, twenty-one and twenty-three eggs respectively. The number of eggs laid per day varied between one and four per female.

The ovipositions recorded in late January, February and March 1939, were, in known cases, extremely short, but it cannot be said that this was due to climatic or seasonal conditions, because at this period a very high percentage of the lice of all stages that were kept in the metal cells died. This could be explained by a very obvious skin reaction of the host animal (see Part II).

Parthenogenesis in *H. eurysternus* has not been noted. On separate occasions, two unfertilized females, which were isolated from other lice, were observed to start oviposition 4 days after emergence. Egg-laying continued at the normal rate until the death of the females 10 and 15 days after oviposition commenced. The forty-five eggs laid were removed and put in an incubator at 30° C. and 70 R.H., but all failed to hatch.

(*e*) *The effect of temperature and humidity on the egg*

Several series of new-laid eggs were kept in glass tubes in airtight jars at different combinations of temperature and humidity and examined daily. The period of incubation varied with the temperature, but not with the humidity. The humidity at 0 and 100 R.H. affected the number of eggs which hatched, but not at 10, 30, 50, 70 and 90 R.H. The emergence of the young larva from the egg was affected by 100 R.H. at any temperature, as the larva pushed off the operculum but died before its legs were out of the egg shell. This was not observed at the lower humidities.

The incubation period, measured in days, increased as the temperature decreased, as follows:

35° C.	30° C.	27·5° C.
9–10	10–11	17–20
av. 10	av. 11	av. 18

At 25° C. no eggs hatched.

Tests were made to determine if partial development of the egg took place at 25° C., by placing the new-laid eggs for periods of 3, 6, 9 and 21 days at 25° C., and then raising the temperature to 30° C. The results showed that no development took place at 25° C., or if any development did occur, it was very slight and was marked by a gradual lethal effect.

(*f*) *Resistance of the egg to low temperatures*

To determine the effect of low temperature on eggs at different stages of development, eggs of *H. eurysternus* were removed from the host on the day of laying, and 3, 6 or 9 days after being laid, exposed to the fluctuating tempera-

ture of the cowstall for 4, 7, 10 or 14 days, and then placed in an incubator at 30° C. 70 R.H., to complete their development. The air temperatures in the cowstall did not rise above 50° F. (10° C.), and usually ranged between 35 and 45° F.

No egg hatched after 21 days' exposure to low temperature. New-laid eggs and eggs allowed 3 and 6 days' development on the cow were all equally susceptible to low temperature, and all but one out of 100 failed to survive 10 days. The eggs removed after 9 days' natural development were more resistant to the subsequent low temperatures, 100% surviving 4 days, 60% 7 days, 5% 10 days and 5% 14 days. The eggs left on the cow, as a control of the natural rate of development, hatched in 11–12 days. Thus, the eggs removed after 9 days on the cow had been allowed 75% of the normal time for development, and the young embryo must have been in an advanced stage of development.

The time taken for these eggs to develop corroborates the findings of the previous section that no development of the embryo takes place below 27·5° C. Thus the threshold of development of the egg of *H. eurysternus* appears to be in the region of 27·5° C.

Preliminary experiments indicated that no eggs of any species of cattle louse will hatch at a moderately low temperature, i.e. the eggs cannot hatch off the host. Bishopp (1921) remarked upon this when discussing *S. capillatus*. McDougall (1923), however, stated that the egg (cattle louse unspecified) remained viable for 3 weeks off the host.

5. SUMMARY

1. The geographical distribution of cattle lice in Britain is recorded in detail. *Bovicola bovis* is the commonest and most widely distributed species in Britain.

2. The incubation period for the eggs was found to be: *Haematopinus eurysternus*, 9–19 days (av. 12); *Bovicola bovis*, 7–10 days (av. 8); *Linognathus vitula*, 10–13 days; *Solenopotes capillatus*, 10–13 days. With eggs of *H. eurysternus* it was found that the higher the minimum air temperature the shorter was the incubation period.

3. In *H. eurysternus* the average length of the instars was: 1st, 4 days; 2nd, 4 days; 3rd, 4 days; pre-oviposition period, 3–4 days. The average time for the complete life cycle, egg to egg, was 28 days.

4. The maximum longevity of *H. eurysternus* on the host was: males, 10 days; females, 16 days. No males or females of *H. eurysternus* survived a starvation period of 72 hr. at 20° C. and R.H. 70 or 0–10° C. and R.H. 70–85; but some nymphs survived this period at 20° C. and R.H. 70, but none survived 96 hr. starvation.

5. The maximum number of eggs recorded for one female was 24; and eggs were laid at the rate of 1–4 a day.

6. The threshold of development of the eggs of *H. eurysternus* appears to be about 27·5° C.

females might have been able to continue egg laying for a greater period if they had been allowed to be refertilized. In two cases an egg-laying female was observed to be in copula.

The maximum number of eggs laid by one female was twenty-four, but it is likely that this number can be exceeded. Three females, which escaped from their cells in November 1938, had laid twenty-one, twenty-one and twenty-three eggs respectively. The number of eggs laid per day varied between one and four per female.

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ture of the cowstall for 4, 7, 10 or 14 days, and then placed in an incubator at 30° C. 70 R.H., to complete their development. The air temperatures in the cowstall did not rise above 50° F. (10° C.), and usually ranged between 35 and 45° F.

No egg hatched after 21 days' exposure to low temperature. New-laid eggs and eggs allowed 3 and 6 days' development on the cow were all equally susceptible to low temperature, and all but one out of 100 failed to survive 10 days. The eggs removed after 9 days' natural development were more resistant to the subsequent low temperatures, 100% surviving 4 days, 60% 7 days, 5% 10 days and 5% 14 days. The eggs left on the cow, as a control of the natural rate of development, hatched in 11–12 days. Thus, the eggs removed after 9 days on the cow had been allowed 75% of the normal time for development, and the young embryo must have been in an advanced stage of development.

The time taken for these eggs to develop corroborates the findings of the previous section that no development of the embryo takes place below 27·5° C. Thus the threshold of development of the egg of *H. eurysternus* appears to be in the region of 27·5° C.

Preliminary experiments indicated that no eggs of any species of cattle louse will hatch at a moderately low temperature, i.e. the eggs cannot hatch off the host. Bishopp (1921) remarked upon this when discussing *S. capillatus*. McDougall (1923), however, stated that the egg (cattle louse unspecified) remained viable for 3 weeks off the host.

5. SUMMARY

1. The geographical distribution of cattle lice in Britain is recorded in detail. *Bovicola bovis* is the commonest and most widely distributed species in Britain.

2. The incubation period for the eggs was found to be: *Haematopinus eurysternus*, 9–19 days (av. 12); *Bovicola bovis*, 7–10 days (av. 8); *Linognathus vitula*, 10–13 days; *Solenopotes capillatus*, 10–13 days. With eggs of *H. eurysternus* it was found that the higher the minimum air temperature the shorter was the incubation period.

3. In *H. eurysternus* the average length of the instars was: 1st, 4 days; 2nd, 4 days; 3rd, 4 days; pre-oviposition period, 3–4 days. The average time for the complete life cycle, egg to egg, was 28 days.

4. The maximum longevity of *H. eurysternus* on the host was: males, 10 days; females, 16 days. No males or females of *H. eurysternus* survived a starvation period of 72 hr. at 20° C. and R.H. 70 or 0–10° C. and R.H. 70–85; but some nymphs survived this period at 20° C. and R.H. 70, but none survived 96 hr. starvation.

5. The maximum number of eggs recorded for one female was 24; and eggs were laid at the rate of 1–4 a day.

6. The threshold of development of the eggs of *H. eurysternus* appears to be about 27·5° C.

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THE CATTLE LICE OF GREAT BRITAIN

PART II. LICE POPULATIONS

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(With 4 Figures in the Text)

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1. Introduction

A previous paper, Craufurd-Benson (1941) gave details of the biology of the cattle lice, with special reference to *Haematopinus eurysternus*. The present paper deals with the seasonal variations of lice populations, and their distribution on the host at different times of the year. The latter is referred to as the regional distribution of lice.

The information has been obtained from two main sources, (*a*) by monthly examinations of cattle at this Research Station, and (*b*) by the distribution of a questionnaire to farmers.

2. Methods

The seasonal and regional distributions of the lice populations were assessed by a monthly examination of ten dairy Shorthorn heifers. In January 1938, the 12 months old heifers had been running in the field since the previous May. The *Bovicola bovis* and *Linognathus vituli* infestations were natural, but the

Haematopinus eurysternus infestations were started artificially in December 1937. At the end of 1938 the ten heifers under examination were replaced by ten 12 months old dairy Shorthorn heifers. In December 1938, the new heifers were found to have considerable infestations of *H. eurysternus*, but had to be artificially infected with *B. bovis*.

The various surface areas of a heifer were defined, and the populations of lice in each area assessed by eye, as the habit of lice to collect in groups rendered it undesirable to adopt a precise method of counting. By experience it became possible to assess the density of any population in one of five categories, and assess the total infestations on a point system, namely, (*a*) very light infestation (1 point), (*b*) light infestation (2 points), (*c*) moderate infestation (3 points), (*d*) heavy infestation (4 points), and (*e*) very heavy infestation (5 points). The presence and number of eggs observed were not used as an indication of the severity of lice, as the egg shells of *H. eurysternus* will remain attached to the hairs for a considerable time after the eggs have hatched, and the apparently unhatched eggs may actually be sterile eggs of considerable age.

The limits of the surface areas of the cow were fixed arbitrarily and the names given to each area are self-explanatory.

3. Seasonal variations

The total lice populations of the ten heifers for each month of the year are shown in Table 1 and Figs. 1 and 2.

Table 1. *Seasonal variation of lice populations*, 1938 *and* 1939

	Total points for month			
Month	*H. eurysternus*	*B. bovis*	*L. vituli*	*S. capillatus*
1938: January	60	150	29	—
February	70	160	32	—
March	66	186	8	—
April	24	22	4	—
May	19	6	0	—
June	17	4	0	—
July	17	4	0	—
August	21	7	0	—
September	24	9	0	—
October	28	11	0	—
November	35	15	0	—
December	58	20	0	—
1939: January	136	11	—	36
February	144	23	—	34
March	98	20	—	25
April	31	26	—	23
May	11	12	—	8
June	2	7	—	1

The records of the variations of the lice populations of all four species of cattle lice show the same general decrease in the populations in the spring. *H. eurysternus* and *B. bovis* populations both increase at the same time in the autumn, and it is probable, although not observed, that *L. vituli* and *S. capillatus* populations would also increase at this time. This suggests that all

four species of lice are reacting to the same factor or combination of factors which control the population densities.

The farmers' observations of the seasonal incidence of lice in general, are given in Figs. 1 and 2 for comparison, where they show a general agreement.

Several of the infestations of *B. bovis* died out during 1938, so that in Fig. 2 a second graph is given showing the infestation level per infected heifer.

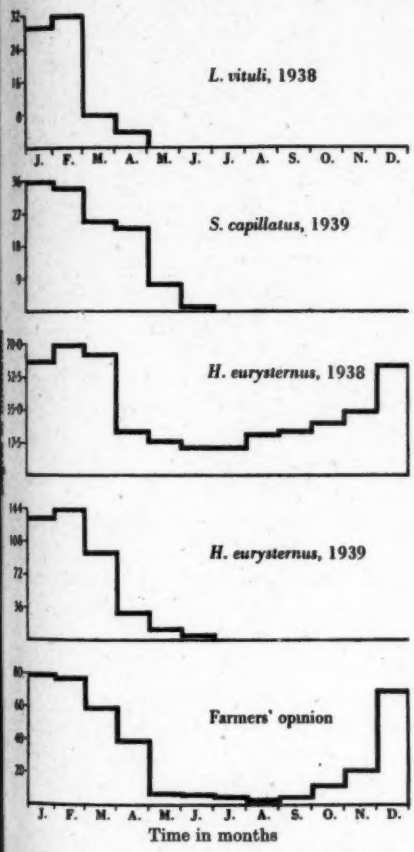

Fig. 1. Seasonal variations of the populations of the sucking lice.

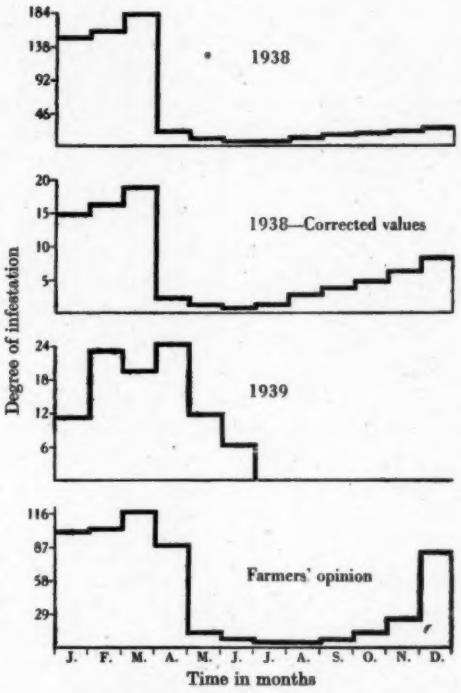

Fig. 2. Seasonal variations of the populations of *B. bovis*.

This gives a more accurate picture of the seasonal increase of the populations in the autumn.

One of the ten heifers in 1938 maintained a high population level of *H. eurysternus* in the summer months. It appears, therefore, that most cattle retain very light infestations in the summer. Some infestations die out altogether, and, in other cases, a high infestation is maintained and the sus-

ceptible animals may be the means of restarting infestations in the following autumn. This last phenomenon has been observed by farmers.

The observations of other workers on the seasonal prevalence of lice can be summarized as follows:

Authority	Seasonal prevalence of lice
Akinschin (1914)	Spring
Cooley & Parker (1916)	In colder months
Imes (1925)	Winter
Lamson (1918)	Winter
Roberts (1938)	Chiefly winter and spring
Shull (1932)	Winter
Underhill (1923)	Commonest towards close of winter months
Walton (1924)	Early spring

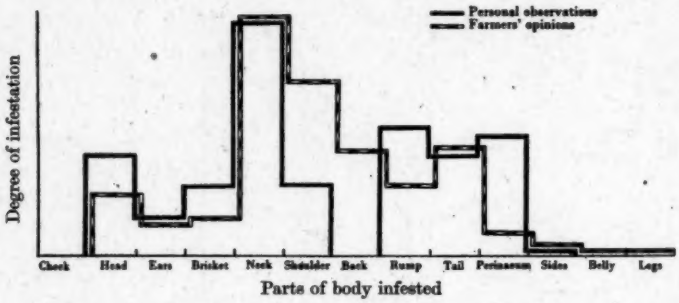

Fig. 3. Typical regional distribution of the sucking lice in the winter.

4. Regional variations on the host

The regional distribution of the lice on the host at different times of the year were assessed at the monthly examinations. In addition, there are eight records of heifers examined at different times of the year, in which every louse of *H. eurysternus* was counted and a chart made of its position on the body, and one count of a *B. bovis* population. The general conclusions from these counts, on sex ratios, etc., have been discussed (Craufurd-Benson, 1941).

The general results of the various examinations are given in Table 2 and a comparison of the typical winter regional distribution of *H. eurysternus* is compared with the farmers' opinions in Fig. 3.

(*a*) *Haematopinus eurysternus*

The first obvious result of the examinations is the differentiation of a population of *H. eurysternus* into breeding colonies and nymphal clusters.

In January the top of the neck is the main breeding area, the females living singly or being clustered together, as many as fifty-eight being recorded in one group. The males are scarce, the male : female ratio varying from 1 : 7 to 1 : 15. The young nymphs are also rare, but a number of third instar nymphs can be observed. Secondary breeding areas can be seen around the base of the

horns, on the brisket and on the tail. If the top of the neck becomes overcrowded the main breeding area extends down the side of the neck, the top section of the side of the neck being used first, and the colony gradually working down to the brisket and dewlap. In cases of extremely heavy infestations any part of the body can be used for breeding, even the legs which are rarely infested. In an average infestation the side of the neck is the principal nymphal area, where the nymphs roam about singly or cluster together in groups. Each of these groups usually has one or more males in attendance. The males are also scattered singly over the side of the neck. The ears, in winter, support a migratory population, consisting usually of only an odd nymph and frequently a male, and rarely is any breeding observed there. The eyelids, and around the eyes in general are favourite places for very young nymphs, but not for the third instar. The shoulders are often used as a breeding area in cases of a heavy infestation, but more usually can be classed as a nymphal area. The back is rarely the habitat of any one stage, but all types can be found here and the lice are usually migrating between the neck and the tail areas. This applies also to the rump area, although breeding colonies and nymphal clusters may be established here as overflows from the tail area. The tail serves as a small and steady breeding area all the year round. Nymphal clusters are often seen on the perinaeum near the anus, where they are protected to some extent by the tail. Adult lice are sometimes found in this region, but rarely breed there. The thigh forms an overflow area from the tail and rump areas, and clusters of egg-laying females often congregate near the ischial process. The sides, belly and legs are rarely infested. Abnormal distributions, such as a heavy scrotal infestation, are sometimes seen, but the usual type of winter distribution in this country is that described above. These observations are in general agreement with the farmers' observations (see Fig. 3).

The gradual shift of the population from the typical winter distribution to the summer areas of infestation, and back again in the autumn is shown in Table 2. The summer areas of infestation can be described briefly as breeding areas on the tail, around the horns and along the inner margin of the ears; the nymphs usually remain near the breeding areas. One farmer stated that lice are found in the summer only round the horns and on the tail; another said that lice seem to congregate along the tips of the ears in spring and early summer.

(*b*) *Linognathus vituli*

There is insufficient evidence to provide a clear picture.

(*c*) *Solenopotes capillatus*

The characteristics of *S. capillatus* infestations is the clustering together of nymphs and adults, mainly on those areas anterior to the shoulders, although some lice have been observed on the shoulders. Repeated attempts to study the life history of this louse have always ended in failure, the lice isolated in

Table 2. *Regional distribution of lice on heifers running in fields*

Month	*Haematopinus eurysternus*	*Bovicola bovis*	*Linognathus vituli*	*Solenopotes capillatus*
January	Mainly on top and side of neck and shoulders infested. Few at tail-head and perinaeum	Lice on back, rump, loins, shoulders and top and side of neck	Lice on top and side of neck and perineal areas	Mainly cheek and side of neck areas infested. Few on brisket
February	Infestations spreading down side of neck	Shoulders and top and side of neck particularly infested	Lice on top and side of neck and perineal areas	Majority of lice on cheek. Brisket more heavily infested; also side of neck infested
March	Ears, base of horns becoming infested. Populations on top of neck and shoulders decreasing while those of tail and rump increasing	Marked decrease in population of top and side of neck. Ribs and belly becoming infested for first time	Lice on perinaeum chiefly	Majority of lice on cheek. Brisket more heavily infested; also side of neck infested
April	Shoulders clean. Top of neck still infested. Base of horns, ears and tail heavily infested	Top and side of neck, shoulders and loins clear. Few on head. Belly, sides, rump and tail infested	Lice on perinaeum only	Main infestation on brisket, few on cheek and side of neck
May	Only few animals maintaining lice on top and side of neck	Belly and sides clear. Two cases of head infestation. Others on rump and tail areas	—	Brisket only infested
June	Base of horns, tips of ears and tail only infested. Ears chiefly	Tail only infested	—	Brisket only infested
July	Base of horns, tips of ears and tail only infested. Ears chiefly	Tail only infested	—	—
August	General increase of population. Three cases of very light infestation on top of neck	Tail infestations increasing	—	—
September	Ear population decreasing. That at base of horns and top of neck increasing	Infestations spreading on to rump from tail area	—	—
October	Top of neck infestation increased considerably. Ears and base of horns populations less. Two cases of rump infestation spreading from tail area	Rump population increasing and lice migrating to thigh areas	—	—
November	Top of neck infestation getting heavy. That of shoulders and side of neck increasing. Rump and perinaeum populations increasing. Ears very lightly infested	Few infestations spreading up loins from rump and tail area	—	—
December	Ears free except for occasional nymph. Infestation of base of horns markedly reduced. Top and side of neck, shoulders well infested. Rump and perinaeum lightly infested	General increase of infestations	—	—

metal cells on the back or rump failing to survive. This may have been due to the inability of the louse to live on the back areas.

The cheek and side of the neck appear to be the main areas for winter infestations, and the brisket for summer infestations.

(*d*) *Bovicola bovis*

The most striking feature of the populations of *B. bovis* is the scarcity of males, a fact that was noticed by Bedford (1932). The examinations also indicated the tendency of populations of *B. bovis* to congregate in breeding colonies and nymphal clusters, and their greater ability, as compared with *H. eurysternus*, to breed on any part of the body. In the new breeding colonies, that is to say, small clusters of adult lice starting a new group in a breeding area, the male : female ratio varied from 1 : 6 to 1 : 20, and in the established breeding areas from 1 : 10 to 1 : 30. The nymphal areas, while being quite distinct, were unlike those of *H. eurysternus* in that many adults were also to be found amongst the nymphs. In these communities the sex ratio of the adults present varied from 1 : 20 up to 1 : 60. The sex ratio for the whole population, in the case observed, was 1 : 24.

In the winter the breeding colonies are chiefly on the sides of the 'top of the neck' area, the side of the neck, shoulders, back, rump and tail. The centres of active breeding are determined by the density of the lice, and the old breeding areas become the nymphal areas, so that all stages of the biting louse are found in the parts specified. The head becomes increasingly infected as the population gets bigger. The sides, thighs and belly are rarely affected. The summer areas of infestation are the head and tail, but it is particularly interesting to note how the population gradually migrates during the spring away from the winter areas of infestation. The sides and belly of the animals are quite heavily infested, but by the summer these areas are free again.

The *B. bovis* populations observed in 1939 did not show the typical winter distribution as the populations were started artificially in December 1938, but the general rise and fall of the total populations was in agreement with the observations of the previous year.

It has been explained that *B. bovis* is usually seen in larger numbers than the sucking lice, and that *B. bovis* causes less individual damage. Thus, for all the different species of lice to have an equally deleterious effect on the cattle, *B. bovis* must be present in much larger numbers than the other lice. Since all the lice start active breeding at the same time in the autumn, as has been shown in a previous section, *B. bovis* would have to breed more rapidly in order to affect the cattle to the same extent, and at the same time, as do the sucking lice. It is a fact that *B. bovis* has a shorter life cycle on the host, but it is not known whether these lice lay more eggs per female than *H. eurysternus* and the others. The implication that *B. bovis* is slower in reaching an effective population density level, *i.e.* one at which it causes damage to the cattle, can be inferred from its later seasonal appearance according to the farmer. It would

suggest that either (*a*) all the lice tend to increase in numbers at approximately the same rate, but that *B. bovis* must be present in such very much larger numbers that it requires a longer period of active breeding before reaching its effective population density, or (*b*) that females of *B. bovis* lay fewer eggs than the females of the sucking lice.

Casual observations on the regional distribution of lice in the winter months have been recorded by several workers, and are in agreement with those given above. The references are as follows:

Cattle lice in general: Akinschin (1914), Lamson (1918), Shull (1932).

Sucking lice in general: Imes (1925), McDougall (1923), Underhill (1923).

Haematopinus eurysternus: Roberts (1938).

Linognathus vituli: Roberts (1938).

Solenopotes capillatus: Bishopp (1921), O'Connor (1932), Roberts (1938).

Bovicola bovis: Imes (1925), Roberts (1938), Thompson (1933), Underhill (1923).

5. Factors affecting the population variations

The monthly average of maximum and minimum screen temperatures, the solar radiation as measured by a vacuum thermometer, the 9 a.m. humidity and the rainfall, as recorded at the Cooper Field Research Station, are given in Fig. 4.

(*a*) *Temperature*

The distribution of cattle lice in Great Britain given in Part I of this series showed that lice infestations were below the average on the east coast of Great Britain, and above the average on the west coast. The east coast in general is subject to lower winter temperatures than the west coast. The general tendency of lice infestations to become more severe, in the opinion of farmers, from north to south, is also a factor which should be considered. These general facts suggest a possible temperature effect, but the seasonal fluctuations of lice populations observed at the Research Station have no relation to the air temperatures.

The skin temperatures of heifers in the field have been recorded at each monthly examination. It was found that the weather at the time of the records affected these temperatures. On warm days, the skin temperatures along the back were higher than normal, and lower on cold days. In general, the forehead and tail skin temperatures were low in comparison with those of the rest of the body. This is interesting, as the tail always harboured lice, while the forehead was only affected in the winter. The 'top of the neck' temperature was taken in a fold of the skin, as the lice usually congregated there. This temperature, and that of the perinaeum were always the highest. The temperatures of the rump, back, shoulder and thigh were usually uniform, but fluctuated according to the air temperature and degree of sunshine. The temperature of the side of the neck was variable, while those of the belly, brisket and legs were low. The ear temperatures fluctuated considerably.

The skin temperatures were rarely below 31° C., and usually fluctuated between 33 and 36° C., so that any part of the body had a suitable skin temperature for breeding purposes at all times of the year.

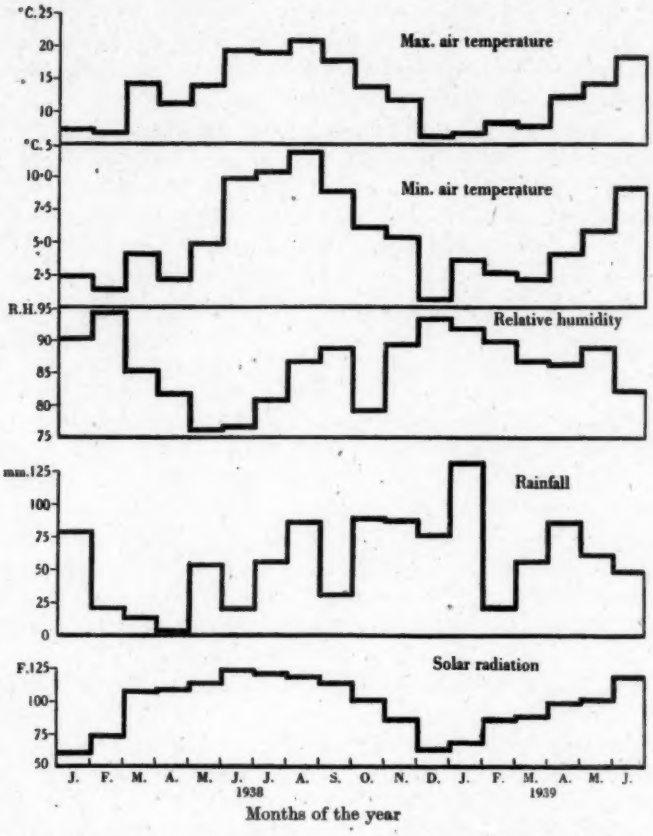

Fig. 4. Meteorological records of the Cooper Field Research Station, 1938–39.

(*b*) *Humidity*

The relative humidity in the coat of cattle has not been ascertained, as no reliable method was evolved.

The air humidity appears to have little correlation with the lice infestations for the humidity fell sharply in March while the lice were still active, and, in the case of *B. bovis*, while the population was definitely increasing. In June 1938, the humidity rose without a corresponding large increase in the populations, and, in October 1938, when the lice were obviously increasing rapidly, the humidity fell sharply.

(*c*) *Rainfall*

The meteorological records show that the degree of rainfall is not correlated with the fluctuations of the lice populations (Figs. 1, 2 and 4).

The importance of rainfall in relation to the quality of the pastures is dealt with in the discussion.

(*d*) *Light*

There are no records at the Cooper Field Research Station for the amount of cloud or for the amount of intensity of daylight, but the solar radiation measured by temperature can be taken as an indication of the degree of light intensity.

There appears to be a close correlation between the infestation levels and the solar radiation temperatures. As the light starts decreasing in July, so the lice start increasing, and in the *H. eurysternus* chart the increase of the lice population is closely allied to the proportional decrease of light. However, in February and March, the light increased while the lice were still very active. In March there was a general dispersal of the populations away from the neck regions which is suggestive of a response to the light. The increase of light in April was negligible, but the lice populations decreased sharply. This apparent anomaly is discussed later.

The records of lice populations in January and February 1939, are of particular interest. The January records show (Tables 1 and 2) the normal lice populations and regional distribution for this period of the year. In February, the lice population increased, as would be expected, but the regional distribution on the host changed. *H. eurysternus* was found in January, on the top of the neck, shoulder, and rump, but in February the lice had decreased in these areas, and the lower regions of the body, the side of the neck and the brisket had become heavily infested. The *Monthly Weather Report* for February 1939 states: "...The excessive sunshine in east and south-east England and parts of the Midlands was noteworthy...." At Harpenden, the nearest Meteorological Station to this Research Station, giving sunshine records, recorded 51 % above the average for the month. The shift of the lice populations from those areas where the light would affect them to areas which were darker in this exceptionally bright month is highly suggestive.

During examinations of lice in the field, it was observed that *B. bovis* always moved away from areas exposed to the light by parting of the hairs; *H. eurysternus* exhibited the same negative phototropism, but was slower to react. Crude qualitative tests in the laboratory corroborated the existence of this negative phototropism.

(*e*) *Coat of the host animal*

The length and thickness of the coat of a cow vary at different times of the year, and a rough estimation of these variations was made at each monthly examination of cattle.

When the lice were most active the coat was thick and long, and in the summer months, when the lice were less active, the coat was thin. During the summer months the lice were found around the horns, on the tips of the ears and on the tail. Each of these areas was covered with thick long hairs, which would afford the lice excellent protection from the direct rays of the sun, and from any marked variations in the air temperature.

The long hairs seen on the tips of the ears in summer disappear in winter. The base of the horns and the tail are well covered with long hairs at all times of the year, and lice were found in these areas all the year round. The usual sites of lice infestations in the winter months have been shown to be the top and sides of the neck, the shoulders and rump. In the winter these areas are covered with a thick coat, but in summer the hair is much thinner. It seems that the general thickness of the coat is of great importance to lice activity. This fact was commented upon by Cooley & Parker (1916).

The length of individual hairs is not of great importance. *B. bovis*, *L. vituli* and *S. capillatus* are usually found in areas where the hairs are short, while the adults of *H. eurysternus* are usually found on the areas with long hairs.

The importance, if any, of the individual hairs is probably due to thickness, for, in general, the long hairs are coarse and thick, while the short hairs are thin. The thickness of the hair might influence the ability of lice to clasp the hair, but this appears unlikely as all ages and species of louse can be found on any part of the body.

The annual shedding of the coat probably also helps to reduce the lice infestations, as numerous eggs, and possibly some of the lice themselves, will be lost as the hairs of the animal fall out (cf. Cooley & Parker, 1916).

The importance of the coat of the host animal can be summarized as follows:

(*a*) The thickness of the coat regulates the temperature gradient between the skin of the animal and the air.

(*b*) The thickness of the coat affords protection to the lice in preventing the access of light.

(*f*) *Skin of the host animal*

It was apparent from personal observations and experiments that the distribution of lice cannot be associated with the thickness of the hide.

Some authors (Underhill, 1923; Shull, 1932; Roberts, 1938) have suggested that lice are affected by the greasiness of the skin of the host because cattle lice are most active in winter when the animal's skin is dry and scaly, whilst in the spring, when the coat is shed and the skin becomes oilier, the lice decrease in numbers.

No reliable method has been evolved of estimating the greasiness of the skin or of the hair, so that no direct evidence bearing on this theory can be given.

Animals which have dry skins are those fed on a high proportion of concentrated foods or on poor quality food. This condition would occur naturally in winter when fodder is scarce. In the spring, the new grass gives a more

succulent type of food, and coincident with this fact is the reduction in the number of lice on the cattle. There is no doubt that animals which are poorly fed get into a low condition of health, which would make them more susceptible to lice infestations. Conversely, well-fed animals given a large ration of concentrated foods should be less susceptible to lice infestation. Cattle lice are, however, often found on these animals, and it is suggested that this is due to the fact of their skin being less greasy than those of grass-fed animals.

Evidence has already been given that the constant feeding of lice confined in the metal cells used for the critical observations, produced, in February and March only, a definite skin reaction. These vesicles ruptured easily and then formed a hard crust. The skin of the necks of animals that have been heavily infested with lice are often crusted and hard, possibly as a result of the formation of a large number of such vesicles. These crusted areas are rarely infested. It is curious that no vesicles are formed as a result of the constant feeding of the lice except in the months of February and March. During these months the natural moulting of the coat takes place. It seems possible that the process of moulting may be coincident with some physiological change in the skin tissues, and that the feeding of the lice may cause obvious and more violent skin reactions while the skin is in this particular physiological state.

After moulting is complete the skin becomes oilier. Further evidence is required before deciding whether the lice decrease as a result of the theoretical physiological change of the skin at the time of moulting, or as a result of a higher oil content of the skin after moulting, or a combination of the two in conjunction with other factors, such as the spatial limitations of 'summer areas' protected from sunlight.

(*g*) *Food of the host animal*

See previous section.

(*h*) *Age of the host animal*

Calves are susceptible to three types of infestation, and less susceptible to *H. eurysternus* infestations, while heifers are susceptible to all four types of infestation. This might explain why farmers see more lice on heifers and, therefore, consider them to be the most susceptible type of animal. It would be fairer to summarize the position by saying (*a*) that calves are housed, so are liable to rapid increases of infestation which are observed because of the frequent handling of them, and (*b*) heifers being susceptible to *H. eurysternus* infestations, which are soon obvious even when there are few lice, are believed to be susceptible types, but, in reality, are probably more resistant than calves. Thus when referring to susceptibility the type of infestation should be specified.

(*i*) *Colour of the host animal*

Numerous observations have been made, but no significant difference was found in the frequency with which any one colour of animal was infested or even the colour of hair to which the lice were attached.

(*j*) *Accessibility of lice to the host animal*

It has been suggested by McDougall (1923) and Imes (1925) that lice are found on those parts of the body where the cow cannot lick them. Observations on cattle tied up in cowsheds indicated that there is no area on which lice are found which the cow cannot either lick or rub.

(*k*) *Housing of cattle*

The questionnaire showed that farmers were almost unanimous in stating that cattle kept in houses were more heavily infested with lice than those animals running in the fields. This has been confirmed by personal observations.

6. DISCUSSION

The most striking feature of the critical work on the life histories was the effect of the air temperature on the incubation period of the eggs. The more accurate laboratory observations on the incubation period of the eggs of *H. eurysternus* showed that the rate of development and hatching of the eggs was entirely influenced by temperature. The humidity appeared to have little effect except at the extreme limits of dry or saturated air. Eggs would not hatch below 27·5° C., and, in addition, all development of the egg appeared to cease below this temperature. The activities and development of *H. eurysternus* did not appear to be so strongly influenced by the air temperature, although some correlation was shown to exist between the rate of oviposition and the general air temperature.

Cattle lice live upon the skin surface of their host, and are sheltered by the coat of the animal from the varying effects of the climate. The length and thickness of the animal's coat will automatically regulate the temperature gradient between the skin and the outer hair. The lice live on the skin, so that they are always in close proximity to their main source of warmth. It has been shown that the skin temperature rarely falls below 30° C., and on most parts of the body where the lice are found the temperature is between 33 and 36° C. The lice which always live on the skin are never subjected to wide fluctuations in temperature, and if, for any cause, the temperature should fall appreciably, the lice are mobile and can move to a more congenial habitat.

The eggs are laid near to the skin, but as they develop they are gradually moved away from the skin by the natural growth of the hair. Thus, they are subjected to a gradually decreasing temperature, and would be more obviously affected by the diurnal fluctuations of temperature. This general theory would suggest that all lice activity is dependent upon the microclimatic temperature, and that this microclimate is, to some extent, influenced by the air temperature.

While temperature is fundamentally the most important factor governing the activity of individual lice, in the warmest times of the year the lice populations are actually very small. Other climatic factors or changes in the conditions on the host, or a combination of these, must so strongly influence

the cattle lice as to override the favourable influence of the temperature factor at this season.

The length and thickness of the coat of the host animal has been shown to vary at different times of the year, the coat being thickest when the lice were most active and vice versa. These variations are a natural response to the variations in climatic conditions.

It has been observed that the microclimatic temperatures in the summer are as high as in winter, so that the lice should be able to breed and live normally throughout the year. However, in the summer months the lice are found at the base of the horns, on the ears and tail areas, which are covered by a dense coat. The dense coat in these areas does not raise the microclimatic temperature above those pertaining in the thin coated areas, because the skin temperatures of the tail and ears, two of the areas with thick coats, are known to be lower than the average for the body surface at all times. The coat appears to be acting as a filter of the light intensity, which supports the previous arguments that the light factor is important in regulating the seasonal activities of lice.

Thus it is believed that the most important of the meteorological factors is light intensity. Laboratory tests and personal observations have substantiated the negative phototropism, and a close correlation has been shown between the seasonal variations of lice populations and the intensity of light. The light intensity increases as the thickness of the coat and the lice populations decrease, and vice versa. Further, in February 1939, records of lice populations showed a change in their regional distribution on the host which was correlated with an exceptionally high light intensity during the month.

It is not suggested that the combined factors of light intensity and thickness of coat are the only ones that regulate the activities of lice, but it is suggested that these combined factors are of very great importance, and do influence the lice populations all the year round.

The other climatic factors, i.e. the rainfall and humidity, did not appear to affect the seasonal variations. It is possible that the rainfall has some importance, as suggested by Roberts (1938) because of its effect on the pastures. Thus rainfall and food are two interdependent factors.

Also the general health of the cattle influences the louse infestations. Whether this be due entirely to the quality of the food, or, as suggested by Roberts (1938) and Underhill (1923), to the nature of the skin secretions as a result of feeding, or both, it is not possible to say. From winter to summer there is a gradual improvement in the quality and nature of the food, the skin becomes greasier, and in the spring there is the annual shedding of the coat which, it has been suggested, may be correlated with a physiological change in the skin. The last statement is based on the obvious skin reaction observed after lice have fed during February and March, and on the crust formations seen on the necks of heavily infested animals.

From the above discussion it may be assumed (*a*) that the interrelated

factors of light intensity and the thickness of the coat of the host animal strongly influence the activities of lice at all times of the year; (*b*) animals in poor health, usually as a result of feeding on poor quality food, are more susceptible to lice infestations than animals in good health; (*c*) that the rainfall is important in countries of periodic rainfall as it improves the quality of the pastures; (*d*) that the quality of the food affects the general health of the animals, and also influences the texture of the skin; and (*e*) that the condition of the skin influences, in some unknown manner, the activities of lice.

It is known, in spite of the scanty records, that cattle lice are found in the majority, if not all, of the cattle rearing countries of the world. It is not so well known which species of lice are found in these countries. The discussion above, on the climatic and physical factors affecting lice populations, would suggest that all species of cattle lice might occur wherever suitable cattle are found, for there does not appear to be any climatic factor limiting any one species of louse to a particular climate. There are records of all the cattle lice from tropical and temperate climates, but none from arctic or antarctic climates.

The distribution of the lice in Great Britain, Craufurd-Benson (1941), did suggest that some factor influenced the distribution of *H. eurysternus*, but not the other species. It is believed that the distribution is affected by the varying farm practice in different areas. There is insufficient evidence to indicate that climatic factors have controlled the distribution, although the distribution in relation to temperature has been commented upon.

The size of the sucking lice of cattle appears to be influenced by the climatic conditions. Roberts (1938) has stated that specimens of *H. eurysternus*, found in tropical regions, are larger than those found in temperate climates. Some specimens of *L. vituli* taken from cattle by Mr W. Downing, F.R.C.V.S., of this Research Station, in Alcopola, Argentine, are considerably larger than those found on the Research Station. Dr Roberts of Yeerongpilly, Queensland, kindly sent me specimens of *S. capillatus* taken from cattle in that country, and these lice are larger than those found in England.

Thus, both *L. vituli* and *S. capillatus*, show size differences in different countries.

7. SUMMARY

1. The seasonal variations and regional distribution of the cattle lice have been studied by monthly examinations of cattle at the Cooper Field Research Station.

2. Additional evidence has been obtained by the distribution of a questionnaire to farmers, the general results of which are included in this paper.

3. The seasonal variations of the populations of the four species of cattle lice in Great Britain are similar. The maximum population density is reached in February and March. In April and May there is a rapid decline until the populations reach their lowest level in June, July and August. In September, the lice start increasing in numbers till they reach their maximum density in February and March.

4. The regional distribution of the population varies in accordance with the seasonal variations of the whole population.

5. The various climatic factors that may affect the lice populations are discussed. It is considered that the light intensity is a factor of major importance in this country.

6. The various factors inherent in the host animal are discussed, and it is suggested that the seasonal fluctuations of the density of the animal's coat are important.

7. The finding of a previous paper (Craufurd-Benson, 1941) that the microclimatic temperature is important as affecting the hatching of eggs is discussed in relation to the variations of lice populations as a whole.

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THE METACERCARIA OF *CERCARIA DORICHA* ROTHS. 1934, OR A CLOSELY RELATED SPECIES

By MIRIAM ROTHSCHILD and NORA G. SPROSTON

(With 2 Figures in the Text)

In 1934 an intensive but unsuccessful search was made for the metacercariae of the Rhodometopa group of cercariae parasitizing *Turritella communis* Lamarck. Attention was concentrated on the vertebrate and invertebrate fauna of the Rame Mud, as *Turritella* has a patchy and circumscribed distribution in the Plymouth neighbourhood and is virtually restricted to a few small areas of ground in this region. The high rate of infection suggested that the metacercariae must occur commonly in some species found there, and the behaviour of the cercariae indicated that a fish served as second intermediate host (Rothschild, 1935, p. 169). This has now been proved.

Two out of a total of six specimens of *Gadus luscus* L.[1] from the Rame Mud, captured on 3 September 1940, were found (N. G. S.) to harbour respectively about one hundred and two oval, thick-walled cysts, in the mesenteric tissue connecting the pyloric caecae. These contained active metacercariae of the Rhodometopa group of cercariae. The absence of pink pigment, shape of the body and size of the excretory granules suggest that the species is *Cercaria doricha*. Without experimental proof, however, it is difficult to assign metacercariae with certainty to any one of several such closely related species.

Remarkably little change apparently occurs after encystment. The tail of course is shed, the penetration glands absorbed and the suckers take on well-defined contours and a more adult form. The small mass of cells representing the anlage of the reproductive organs, situated anterior to the ventral sucker, becomes more compact and forms a hook-shaped mass. In other respects the metacercariae resemble the cercariae in all essentials. The large dendritic excretory vesicle, by far the most characteristic and conspicuous feature of this group, is similar in all details. Even in preserved material the post acetabular transverse connection between the forks of the Y can be made out.

The excysted metacercariae were fixed in Bouin Duboscq under light pressure of a cover-slip and stained with Delafield's Haematoxylin (Figs. 1, 2). Measurements of 14 specimens are given in Table 1. They are rather smaller than living cercariae (except for the suckers), but this is no doubt due to fixation and is probably more apparent than real. The prepharynx is only present in some specimens. Although not mentioned in the text of the original description, this feature was occasionally noted in *C. doricha*, but it was then

[1] Two specimens of *Gadus merlangus* (out of a total of fifty specimens dissected from various localities) were each found to harbour three apparently similar cysts in the same site. The excysted metacercariae measured, proved to be smaller than those from *G. luscus*.

thought to be an artefact due to unnatural pressure. It seems likely that the adult worm will possess a prepharynx.

In 1932 and 1933 attempts were made to induce these cercariae to penetrate *Gadus merlangus* through the skin, but without success. Possibly the cercariae

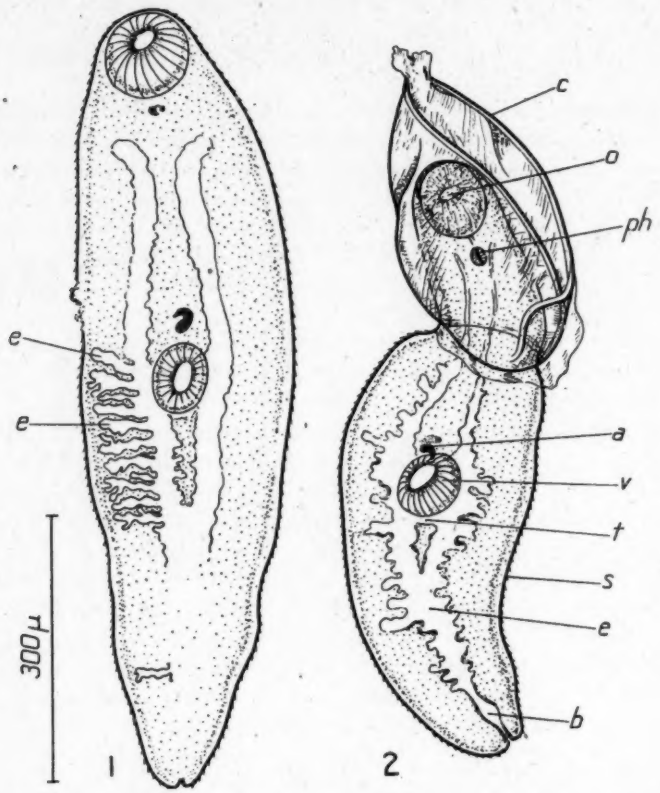

Fig. 1. Excysted metacercaria. Fig. 2. Metacercaria issuing from the cysts. *a*, anlage of reproductive organs; *b*, bladder; *c*, cyst; *e*, excretory vesicle; *o*, oral sucker; *ph*, pharynx; *s*, spines; *t*, transverse commissure connecting branches of Y-shaped excretory vesicle; *v*, ventral sucker.

Note. The portions of excretory vesicle shown in Figs. 1 and 2 are the only parts visible on these actual specimens. In the living metacercaria the granules in the vesicle give it a dark appearance.

are swallowed and make their way through the wall of the gut from the interior. The behaviour of *Cercaria doricha* suggests that *Gadus luscus* is the natural host as it is more often a bottom feeder than *G. merlangus*.

The search for the final host should now be considerably narrowed as there are relatively few fish which both consistently prey upon *G. merlangus* and

Table 1. *Measurements (in microns) of fourteen mounted specimens and cysts*

															Means
Body:															
Length	758	783	790	899	942	955	965	975	990	998	1017	1062	1083	1107	952
Width	234	274	260	239	338	280	285	345	363	305	285	372	267	288	295
Ratio:															
$\frac{\text{Length}}{\text{Width}}$ (%)	323	287	304	375	271	341	339	233	273	326	356	285	405	399	323
Oral sucker:															
Length	104	115	99	94	107	114	112	110	110	109	110	116	102	120	109
Width	115	127	110	110	114	99	117	119	112	117	120	129	107	115	115
Ventral sucker:															
Length	86	99	87	84	100	104	112	104	89	89	99	99	102	110	98
Width	97	71	94	84	87	96	84	106	84	86	68	117	86	104	90
Ratio*															
$\frac{\text{Oral sucker}}{\text{Ventral sucker}}$ (%)	121	142	115	121	117	106	116	109	129	130	139	113	111	109	118
Forebody†	376	375	368	425	460	462	417	428	523	468	428	485	488	527	445
Ratio:															
$\frac{\text{Length of body}}{\text{Length of forebody}}$ (%)	202	209	215	211	205	207	231	228	189	213	238	220	222	210	213
Cysts:															
Length	527	508	480	465	449	445	445	445	436	434	434	412	403	395	448
Width	404	362	263	356	341	362	346	296	363	356	294	314	279	345	334

* Ratio calculated from mean diameter of ventral and oral suckers.

† Distance between anterior extremity and anterior margin of ventral sucker.

G. luscus and are common over the Rame Mud. The most obvious are the angler fish (*Lophius piscatorius* L.), the turbot (*Rhombus maximus* L.), large specimens of monk fish, *Rhina squatina* (L.), large pollack (*Gadus pollachius* L.), any large rays (*Raja* spp.) and the larger specimens of gurnards (*Trigla* spp.). In their early stages the whiting, of course, falls victim to a much wider range of predators. The fact that the intermediate host is found at a depth of 20 fathoms or more, and is yet commonly infected with Rhodometopa cercariae makes it exceedingly unlikely that the final host is a bird. But for the war, which unfortunately precludes the collection of *Turritella*, this discovery would open up a vista of charming experiments.

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